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Interaction between tree diversity and water availability on nutrient cycling in forests

Sous la direction de Dr. Laurent AUGUSTO,
de Dr. Nicolas FANIN, et de Prof. Dr. Alison D. MUNSON

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Résumé

Les forêts mélangées sont l'une des principales options de gestion pour stabiliser la production de biomasse forestière tout en réduisant l'impact du stress hydrique de plus en plus fréquent avec les changements globaux. Cependant, les effets interactifs entre diversité des espèces d'arbres et disponibilité en eau ont rarement été étudiés *in situ*, et encore moins en étudiant le fonctionnement du sol. Dans cette thèse, nous avons cherché à déterminer si les effets positifs de la diversité des espèces d'arbres sur le recyclage des nutriments étaient modulés par la disponibilité en eau. Pour répondre à cette question, deux sites appartenant au réseau mondial de diversité des arbres (TreeDivNet) ont été étudiés : ORPHEE dans le sud-ouest de la France et IDENT-SSM à Sault Ste. Marie au Canada. Ces deux sites expérimentaux manipulent la diversité des arbres (richesse des espèces d'arbres) et la disponibilité en eau (irrigation dans les deux cas, avec exclusion partielle des pluies à IDENT-SSM). Les travaux sont divisés en quatre chapitres : (1) la complémentarité spatiale verticale de la disponibilité des nutriments du sol, (2) la dépolymérisation de l'azote organique du sol, (3) la complémentarité temporelle dans les prélèvements de nutriments et les processus du sol, et (4) l'efficience d'utilisation des nutriments par les arbres.

En examinant le cycle des nutriments dans les sols, nous avons constaté qu'une forte disponibilité en eau augmentait la dégradation de la matière organique (par les enzymes extracellulaires et la dépolymérisation des protéines), tandis qu'elle diminuait la minéralisation nette des nutriments. Un effet du mélange d'espèces d'arbres n'a été observé que dans la couche intermédiaire du sol, où l'on a trouvé une teneur en matières organiques et des activités enzymatiques plus élevées que dans les monocultures. Au cours de l'année, des différences dans la disponibilité des nutriments n'étaient visibles que lorsque la disponibilité en eau était suffisante : il y avait plus d'azote biodisponible dans les parcelles de pins en monoculture, tandis que le phosphore était plus important dans les parcelles de bouleaux en monoculture, par rapport aux autres traitements d'espèces. Malgré les variations saisonnières, ces tendances sont restées constantes entre les périodes de dormance et de végétation. En étudiant expérimentalement l'absorption des nutriments, nous avons observé une asynchronie de l'absorption lors du débourrement de la végétation:

à ce stade phénologique, l'espèce sempervirente (pin) a pût prélever plus tôt que l'espèce décidue (bouleau), avec respectivement un avantage et un désavantage pour l'absorption des nutriments dans les parcelles mélangées. En ce qui concerne l'efficience d'utilisation des nutriments (NutUE), nous avons constaté que l'identité des espèces était un facteur plus important que la diversité des espèces. Bien que l'augmentation de diversité ait eu tendance à accroître la productivité aérienne et la biomasse de la litière, la dominance de certaines espèces dans les mélanges était le principal facteur contrôlant la NutUE (le bouleau sur le pin à IDENT-SSM et le pin sur le bouleau à ORPHEE). Ainsi, les processus biogéochimiques de la couche superficielle du sol ont été déterminés par la disponibilité en eau, les processus du sol intermédiaire par le mélange des espèces, et l'efficience d'utilisation des nutriments a été déterminée par l'identité des espèces plutôt que par le mélange. A l'inverse de l'effet prononcé des facteurs pris individuellement, nous n'avons pas observé d'effet interactif entre ceux-ci. Sur la base de l'ensemble de nos résultats, nous concluons que chacun des facteurs étudiés (disponibilité en eau, diversité des arbres et identité des espèces) joue un rôle significatif sur le recyclage des nutriments, même s'il n'y avait pas d'effets interactifs.

Mots-clés : biodiversité, changements globaux, cycles biogéochimiques, disponibilité des nutriments du sol, absorption des nutriments, efficience d'utilisation des nutriments

Abstract

Mixed forests are one of the main management strategies to stabilize forest biomass production while reducing the impact of water stress, which is becoming more frequent as a result of global changes. However, the interactive effects between tree diversity and water availability have rarely been studied *in situ*, let alone while studying soil functioning. In this thesis, we sought to determine whether the positive effects of tree species diversity on nutrient cycling in forests were modulated by water availability. To answer this question, two sites belonging to the global tree diversity network (TreeDivNet) were studied: ORPHEE in southwestern France and IDENT-SSM in Sault Ste. Marie, Canada. These two experimental sites manipulate tree diversity (tree species richness) and water availability (irrigation in both cases, with partial rain exclusion at IDENT-SSM). The work is divided into four chapters: (1) vertical spatial complementarity of soil nutrient availability, (2) soil organic nitrogen depolymerization processes, (3) temporal complementarity of nutrient uptake and soil nutrient processes, and (4) nutrient use efficiency by trees.

When looking at belowground nutrient cycling, we found that high water availability increased organic matter breakdown (by extracellular enzymes and protein depolymerization), while it decreased net nutrient mineralization. An effect of mixing tree species was only detected in the mid soil layer, where we observed a higher amount of organic matter and enzyme activities compared to the monocultures. Throughout the year, differences in nutrient availability were only visible when water availability was sufficient: more bioavailable nitrogen was produced in the pine monocultures, while more phosphorus was produced in the birch monocultures, compared to the other species treatments. Despite seasonal variation, these trends remained consistent between the dormant and vegetative periods. When experimentally investigating nutrient uptake, we observed an asynchrony of uptake during budburst: at this phenological stage, the evergreen species (pine) was able to take up nutrients earlier than the deciduous species (birch), with an advantage and a disadvantage respectively for nitrogen uptake in the mixed plots. Finally, we found that species identity was a stronger driver than species diversity for aboveground nutrient use efficiency (NutUE). Although increasing tree species tended to increase aboveground

productivity and litterfall biomass, certain species dominance drove NutUE trends (i.e. birch dominance over pine at IDENT-SSM and pine over birch at ORPHEE). In summary, surface soil biogeochemical processes were driven by water availability, intermediate soil processes by species mixing, and nutrient use efficiency was driven by species identity rather than mixing. In contrast to the pronounced effect of individual factors, we did not observe an interactive effect between them. Based on all of our results, we conclude that each of the factors studied (water availability, tree diversity, and species identity) had an important role in nutrient cycling, even though there were no interactive effects.

Keywords: biodiversity, climate change, biogeochemical cycles, soil nutrient availability, nutrient uptake, nutrient use efficiency

Résumé substantiel

Les forêts mélangées sont l'une des principales options de gestion pour stabiliser la production de biomasse tout en réduisant l'impact du stress hydrique, qui devient plus fréquent et sévère avec les changements globaux. Cependant, les effets interactifs entre diversité des arbres et disponibilité en eau ont rarement été étudiés *in situ*, et encore moins sur le fonctionnement du sol. Dans cette thèse, nous avons cherché à déterminer si les effets positifs de la diversité des espèces d'arbres sur le recyclage des nutriments en forêt étaient modulés par la disponibilité en eau. Pour répondre à cette question de recherche, deux sites appartenant au réseau mondial de diversité des arbres (TreeDivNet) ont été étudiés : ORPHEE dans le sud-ouest de la France et IDENT-SSM à Sault Ste. Marie au Canada. Ces deux sites expérimentaux manipulent la diversité des arbres (richesse spécifique des arbres) et la disponibilité en eau (irrigation dans les deux cas, avec un système d'exclusion des pluies supplémentaire à IDENT-SSM). À ORPHEE, nous avons comparé des peuplements de monoculture de pin maritime (*Pinus pinaster*) et de bouleau argenté (*Betula pendula*) avec des peuplements mixtes de ces mêmes espèces, ainsi que leurs paires irriguées. Dans le second site au Canada, nous avons étudié cinq espèces uniques (*Pinus strobus, Betula papyrifera, Acer saccharum, Larix laricina, Quercus rubra*) ainsi que plusieurs mélanges de deux et quatre espèces, dans des blocs irrigués et d'exclusion des pluies. Le site ORPHEE a été étudié dans les quatre chapitres de cette thèse, alors qu'IDENT-SSM n'a été inclus que dans le dernier chapitre. Les quatre chapitres portent sur les thèmes suivants: (1) la complémentarité spatiale verticale des nutriments et de l'activité microbienne, (2) les processus de dépolymérisation de l'azote organique du sol, (3) la complémentarité temporelle dans les prélèvements de nutriments et les processus du sol, et (4) l'efficience d'utilisation des nutriments par les arbres.

L'objectif principal du **Chapitre 1** était d'étudier l'effet interactif entre la diversité des arbres et la disponibilité en eau sur les nutriments disponibles et les activités des enzymes extracellulaires du sol (EEA) le long du profil du sol. Pour ce faire, nous avons échantillonné des sols à ORPHEE en mars 2018 : des carottes de sol ont été prélevées dans 3 traitements de composition d'espèces (bouleau pur, pin pur, mélange bouleau-pin), chacun répété dans 4 blocs de disponibilité en eau élevée (*via* irrigation) et 4 blocs de disponibilité

en eau faible (soumis aux étés secs du sud-ouest de la France). Chaque carotte de sol a été divisée en 5 couches de sol (0-5, 5-15, 15-30, 30-60, et 60-90 cm), dans lesquelles nous avons mesurés les nutriments disponibles et des activités enzymatiques extracellulaires liées au carbone (C_{enz}), à l'azote (N_{enz}) et au phosphore (P_{enz}). Les résultats de l'expérience ont montré que les EEAs du sol dépendaient directement de la composition des espèces d'arbres et des conditions hydriques en interaction avec la profondeur du sol. Tout d'abord, nous avons constaté un effet positif du mélange de bouleaux et de pins sur les C_{enz} et N_{enz} à une profondeur de sol intermédiaire (horizon de sol de 15-30 cm). Deuxièmement, l'augmentation de la disponibilité en eau a augmenté les EEAs liées au phosphore (P) principalement dans les couches supérieures du sol (0 à 30 cm). Cependant, nous n'avons pas trouvé d'effet interactif significatif entre la diversité des arbres et la disponibilité en eau sur les EEAs, ce qui sous-entend que l'effet négatif d'une disponibilité en eau plus faible ne peut pas être compensé par l'effet positif du mélange des espèces d'arbres. De plus, nous avons trouvé une forte corrélation positive entre le P disponible et le rapport C_{enz}:P_{enz}. Cela implique que lorsque la disponibilité du P est élevée, les microorganismes sont fortement limités par le C, ce qui les incite à produire plus d'enzymes liées au C pour accéder à l'énergie produite par la décomposition de la matière organique. Cette étude a confirmé qu'il existe une complémentarité spatiale de l'activité des microorganismes dans la couche intermédiaire sur des podzols tempérés landais, mais que cet effet n'a pas été affecté par les changements de disponibilité en eau.

Étant donné que plus de 50% du N total du sol est représenté par des structures peptidiques, la dépolymérisation des protéines en oligopeptides et en acides aminés libres (c'est-à-dire le processus précédant la minéralisation en azote minéral), détermine le recyclage de N du sol et la quantité d'azote disponible pour les plantes et les microorganismes. Peu d'études se sont penchées sur les taux bruts de dépolymérisation des protéines, et aucune n'a étudié comment le mélange d'espèces d'arbres pouvait affecter ce processus. De plus, nous avons voulu étudier comment les différences de disponibilité en eau pouvaient avoir un impact sur les effets potentiels du mélange, ce qui a été l'objectif du **Chapitre 2**. En juillet 2020, nous avons prélevé trois carottes de sol (couche de 0 à 15 cm) par parcelle sur le site de terrain ORPHEE (bouleau, pin, bouleau + pin), chaqune étant répliquée 3 fois en fonction de deux niveaux de disponibilité en eau: irrigué ou non-irrigué. Le C et N de la biomasse microbienne ont été mesurés sur des échantillons de sol frais en utilisant la méthode de fumigation-extraction. Pour mesurer les taux bruts de la dépolymérisation des protéines, nous avons utilisé la technique de dilution des pools isotopiques et une nouvelle méthode à haut débit de la quantification du ^{15}N dans les acides aminés, en collaboration avec l'équipe 'Terrestrial Ecosystem Research' de l'Université de Vienne en Autriche. Globalement, nous n'avons pas trouvé d'effet significatif du mélange de bouleau et de pin sur la dépolymérisation des protéines, ni sur la biomasse microbienne. Cependant, une disponibilité en eau élevée a conduit à une augmentation de la dépolymérisation des

protéines, sans effet significatif sur la biomasse microbienne N ou sur l'activité spécifique. Ces résultats suggèrent que l'effet positif d'une disponibilité en eau élevée sur cette dépolymérisation était principalement dû à une augmentation de la solubilité des substrats et/ou d'une plus grande rencontre entre substrats et enzymes. Bien que nous n'ayons pas trouvé d'effets interactifs significatifs entre le mélange d'espèces d'arbres et la disponibilité en eau sur les processus de décomposition de l'azote organique du sol, quelques tendances sont ressorties, tel qu'une augmentation des processes en mélange sous une disponibilité en eau élevée. Dans l'ensemble, la disponibilité en eau a été le facteur le plus important, augmentant les taux de dépolymérisation du N organique du sol dans toutes les parcelles de composition des espèces.

Dans le **Chapitre 3**, nous avons exploré la complémentarité temporelle du mélange de bouleau et de pin sur plusieurs processus nutritifs souterrains, avec une disponibilité en eau faible et élevée dans la plantation expérimentale ORPHEE. La complémentarité temporelle peut se produire avec des taux variables de libération des nutriments dans les peuplements mixtes par rapport aux peuplements en monoculture tout au long de la saison de croissance, en raison des différences de qualité et de récalcitrance de la litière. Un autre mécanisme saisonnier serait une différence dans la synchronisation de l'activité et de l'absorption des nutriments entre les espèces à feuilles caduques et les conifères, l'activité de ces derniers pouvant commencer plus tôt en raison d'un feuillage présent toute l'année. Notre étude était basée sur un dispositif employant différentes méthodes complémentaires afin d'étudier différents aspects dans la temporalité du cycle des nutriments : des résines échangeuses d'ions ont été utilisées pour estimer la disponibilité des nutriments du sol, la méthode d'incubation du sol *in situ* de Raison pour les taux de minéralisation nets, et deux campagnes de marquage du sol au ^{15}N (débourrement et pic de phénologie). Nous avons étudié le bouleau pur, le pin pur et le mélange bouleau-pin dans des conditions de disponibilité en eau faible et élevée (3 blocs répétés chacun). Une disponibilité en eau plus élevée a entraîné des différences dans la disponibilité des nutriments du sol (mesurée à 8 cm de profondeur) : les peuplements de pin pur et de bouleau pur étaient caractérisés par un taux plus élevé d'azote minéral du sol et un taux plus élevé de phosphore disponible, respectivement. Cependant, les taux de minéralisation nets étaient similaires entre les traitements de composition des espèces, mais ont été impactés négativement par une disponibilité en eau élevée. Enfin, les espèces au sein du mélange présentaient des dynamiques d'absorption différentes, influencées par la croissance et le couvert végétal, mais pas par la disponibilité en eau. Cette asynchronie dans l'absorption des nutriments était présente au débourrement, mais disparaissait ensuite au pic de la phénologie en été. Ce chapitre conclut que le timing des demandes et de l'absorption des nutriments par les espèces au sein d'un mélange peut être un facteur plus important que la minéralisation nette pour la disponibilité des nutriments du sol dans les jeunes plantations de forêts mixtes. Dans l'ensemble, la minéralisation nette et la disponibilité des éléments nutritifs du sol ont

été plus fortement déterminées par la disponibilité en eau que par le mélange des espèces d'arbres.

Le **Chapitre 4** met l'accent sur l'efficience d'utilisation des nutriments (NutUE), la dernière étape pour compléter notre étude du recyclage des nutriments dans les forêts. La NutUE correspond à l'efficience par laquelle les espèces d'arbres utilisent les nutriments disponibles pour produire leur biomasse. Dans ce chapitre, nous nous sommes demandé comment l'approvisionnement en eau pouvait influencer l'effet de la richesse spécifique et de l'identité des espèces sur la NutUE au niveau du peuplement. Nous avons mesuré la production annuelle de masse de litière, la concentration de certains nutriments dans les feuilles vertes et la litière, ainsi que la productivité primaire nette aérienne (ANPP) de plusieurs espèces d'arbres en monoculture et en mélange sur les sites expérimentaux d'ORPHEE et IDENT-SSM. Nous avons ensuite calculé la NutUE à l'échelle du peuplement en divisant l'ANPP par le flux de macronutriments induit par la chute de la litière. Nous avons également estimé la disponibilité des nutriments du sol en installant des résines échangeuses d'ions, et calculé l'efficience de la résorption des nutriments (NutRE) en utilisant à la fois la concentration en nutriments des feuilles vertes et de la litière, ainsi qu'un facteur de correction de la masse des feuilles. La NutRE est la capacité des arbres à retransloquer de manière efficiente les nutriments foliaires vers les tissus en développement ou les sites de stockage avant l'abscission des feuilles. Nous avons trouvé des effets significatifs de la richesse spécifique (SR) et de la disponibilité en eau sur la NutUE. Cependant, ces effets étaient faibles et variés en fonction du site d'étude et de l'élément considéré. Les effets de l'identité des espèces se sont avérés plus explicatifs en examinant des parcelles de bouleaux et de pins sur les deux sites. À ORPHEE, l'efficience d'utilisation de l'azote (NUE) et l'efficience d'utilisation du phosphore (PUE) étaient significativement plus grandes dans le mélange pin-bouleau que dans chaque monoculture. Cela était dû à une augmentation de l'ANPP ainsi qu'à une diminution des nutriments de la litière dans la parcelle à deux espèces, en raison de la dominance du pin dans ce mélange. Dans les parcelles plus jeunes et plus denses d'IDENT-SSM, le bouleau était dominant et a entraîné un effet positif du mélange sur l'ANPP et les concentrations de nutriments de la litière dans les mélanges pin-bouleau. Ceci a conduit à un effet neutre du mélange sur la NUE et la PUE. Dans l'ensemble, les effets du mélange étaient semblables dans les traitements à faible et à forte teneur en eau, ce qui suggère que la composition des espèces est plus importante que la disponibilité en eau pour la NUE au niveau du peuplement dans les jeunes communautés forestières.

Trois **conclusions** principales de cette thèse peuvent être soulignées. Premièrement, la disponibilité en eau était le facteur le plus important pour les processus liés aux nutriments du sol dans nos sites expérimentaux, avec une faible disponibilité en eau menant à une diminution de la quantité de matière organique et une dégradation plus lente, et une immo-

bilisation microbienne plus élevée. Cela a des implications négatives pour le recyclage des nutriments dans un climat futur, qui devrait connaître des périodes plus longues et plus sèches de disponibilité en eau réduite. Dans le sol, l'effet de la composition des espèces était à la fois limité dans l'espace et dans le temps : il y avait un effet positif du mélange sur la matière organique et l'activité des microorganismes dans la couche intermédiaire du sol, et il y avait un effet gagnant-perdant du mélange sur le pin par rapport au bouleau lors de l'absorption de nutriments au débourrement. Il n'y avait pas non plus d'effet interactif du mélange et de la disponibilité en eau, ce qui suggère que le mélange des espèces d'arbres ne compense pas toujours les effets négatifs de la disponibilité réduite en eau sur les processus nutritifs du sol. Pour la partie aérienne du cycle des nutriments, la composition des espèces a été déterminante, et la disponibilité en eau a eu peu d'effet. La dominance des espèces, influencée par l'âge et la densité du peuplement, a déterminé la productivité et les flux de nutriments de la litière, et finalement l'efficience d'utilisation des nutriments au niveau du peuplement. Il est important de tenir compte de cette séparation à la fois spatiale et temporelle, et de la non-interaction entre la diversité des arbres et la disponibilité en eau lorsque l'on tire des conclusions sur ces facteurs.

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Au milieu de l'hiver,
j'apprenais enfin
qu'il y avait en moi
un été invincible.

Albert Camus, L'Eté (1954)

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Preface

This thesis is organized into four chapters written as scientific papers in English. For each chapter, I established the research objectives and hypotheses, organized the experimental designs, field work, and laboratory work, managed the data, analyzed the data statistically, generated all figures, and wrote the first drafts. I am first author on all four papers.

Chapter 1 – "Effect of a tree mixture and water availability on soil nutrients and extracellular enzyme activities along the soil profile in an experimental forest" was published in the journal *Soil Biology and Biochemistry* (148, 107864) in June 2021. The co-authors are Laurent Augusto, Lucie Bon, Adèle Courbineau, Andreas Altinalmazis-Kondylis, Sylvie Milin, Mark R. Bakker, Hervé Jactel, and Nicolas Fanin.

Chapter 2 – "High water availability stimulates soil organic nitrogen breakdown, while tree mixing has little effect" is in preparation for submission. The co-authors are Laurent Augusto, Yé Tian, Wolfgang Wanek, and Nicolas Fanin.

Chapter 3 – "Tree species composition and nutrient uptake drive soil nutrient availability, even under contrasted levels of water availability" is in preparation for submission. The co-authors are Nicolas Fanin, Alison D. Munson, Catherine Lambrot, Laura Scolan, Pierre Trichet, and Laurent Augusto.

Chapter 4 – "Tree species dominance drives nutrient use efficiency in young mixed-species plantations, at both high and low water availability" is currently under review. The co-authors are Nicolas Fanin, William C. Parker, Mark R. Bakker, Ariane Belleau, Céline Meredieu, Laurent Augusto, and Alison D. Munson.

My work was supervised by my thesis supervisors, Laurent Augusto, Nicolas, Fanin and Alison D. Munson, who are included in the papers as co-authors. The following co-authors helped with experimental design, sampling, and editing of the manuscripts: Andreas Altinalmazis-Kondylis, Mark R. Bakker, Hervé Jactel, Céline Meredieu, William C. Parker, and Wolfgang Wanek. The following co-authors helped with sampling, laboratory work, analysis, and editing of the manuscripts: Ariane Belleau, Lucie Bon, Adèle Courbineau, Catherine Lambrot, Sylvie Milin, Laura Scolan, Yé Tian, and Pierre Trichet.

Introduction

A GENERAL CONTEXT

Forests are an important terrestrial ecosystem, which provide a multitude of ecosystem functions and services. Yet, they are increasingly impacted by global changes such as a reduction in precipitation (IPCC 2014; Schwalm et al. 2012)[1]. Reductions in water availability, leading to drought, may have various negative impacts on forest functioning, such as reducing conductance and thus biomass (He and Dijkstra, 2014), microbial biomass (Brockett et al. 2012) and activity (Sardans and Peñuelas 2005), nutrient availability (Augusto et al. 2017; Bengtson et al. 2005), and overall nutrient cycling (Schlesinger et al. 2016). Increasing tree diversity has been found to increase productivity in comparison to monocultures (Gamfeldt et al. 2013), and is an option to mitigate negative effects of reduced soil water availability, such as during droughts. Mixed stands have been found to be more stable to climate fluctuations than monocultures, due to overyielding, species asynchrony and favorable species interactions (Jucker et al. 2014a; Kardol et al. 2018; Morin et al. 2014). While most studies have focused on the effect of tree diversity on aboveground aspects of forest functioning, only a few studies have investigated these effects on belowground functioning (Alberti et al. 2017; Brassard et al. 2013; Ma and Chen 2016), and we know little about how reduced water availability may affect biodiversity benefits on nutrient cycling. **The purpose of this thesis is thus to investigate the interactive effects of tree diversity and water availability on the different steps of nutrient cycling in forests**.

In order to present the different aspects of the thesis, an introductory general context is detailed below, and divided into two main parts. In the first part, I give a review of important concepts as a basis for the thesis (two sections dedicated to [A.1] reduced precipitation due to global changes and [A.2] forest functioning). Then, in the second part, I review the literature more specifically linked to the research question of this thesis, grouped in three sections [A.3, A.4, A.5]: the effect of tree diversity, the effect of water availability, and the effect of their interaction on nutrient cycling in forests. These five

[1]References can be found in the final Bibliography section

sections will give an overview of the various themes addressed during the thesis, after which I will present the main objectives and hypothesis (Section B), and the general material and methods of the different chapters (Section C).

A.1 Global changes

A.1.1 Changes to the climate

Globally, temperatures and CO_2 are rising, and seasonal precipitation patterns are changing, with certain regions with heavier rains in winter and some with more frequent and severe droughts during the summer (IPCC 2014). Figure A.1 presents the predicted changes in average temperature and precipitation for the years 2081-2100, from the reference years 1986-2005, based on two Representative Concentration Pathways (RCPs). These are used to make projections to describe different 21st century pathways of several climate change drivers, such as greenhouse gas (GHG) emissions and atmospheric concentrations, air pollutant emissions and land use. In both the stringent mitigation scenario (RCP2.6), that aims to keep global warming below 2°C above the pre-industrial average temperatures, and the more extreme scenario (RCP8.5), in which no additional efforts are made to constrain emissions, we can see that temperatures are expected to globally rise, and that precipitation will decrease in some areas, and increase in others, both of which may have negative impacts. Droughts are not only predicted to be more frequent (McDowell et al. 2018), but they are expected to last longer. When looking at predicted changes at the European level (Fig. A.2, Lindner et al. 2014), there is predicted to be a strong increase in annual maximum continuous dry days in the years 2070-2099 compared to mean maximum dry days in the 1961-1990 period. This can have substantial negative consequences on plant growth and forest productivity, as drought may cause a reduction in primary productivity and in carbon sequestration (Schwalm et al. 2012). **In this thesis, the studies will focus on the impact of reduced water availability, such as during drought, on forest functioning**.

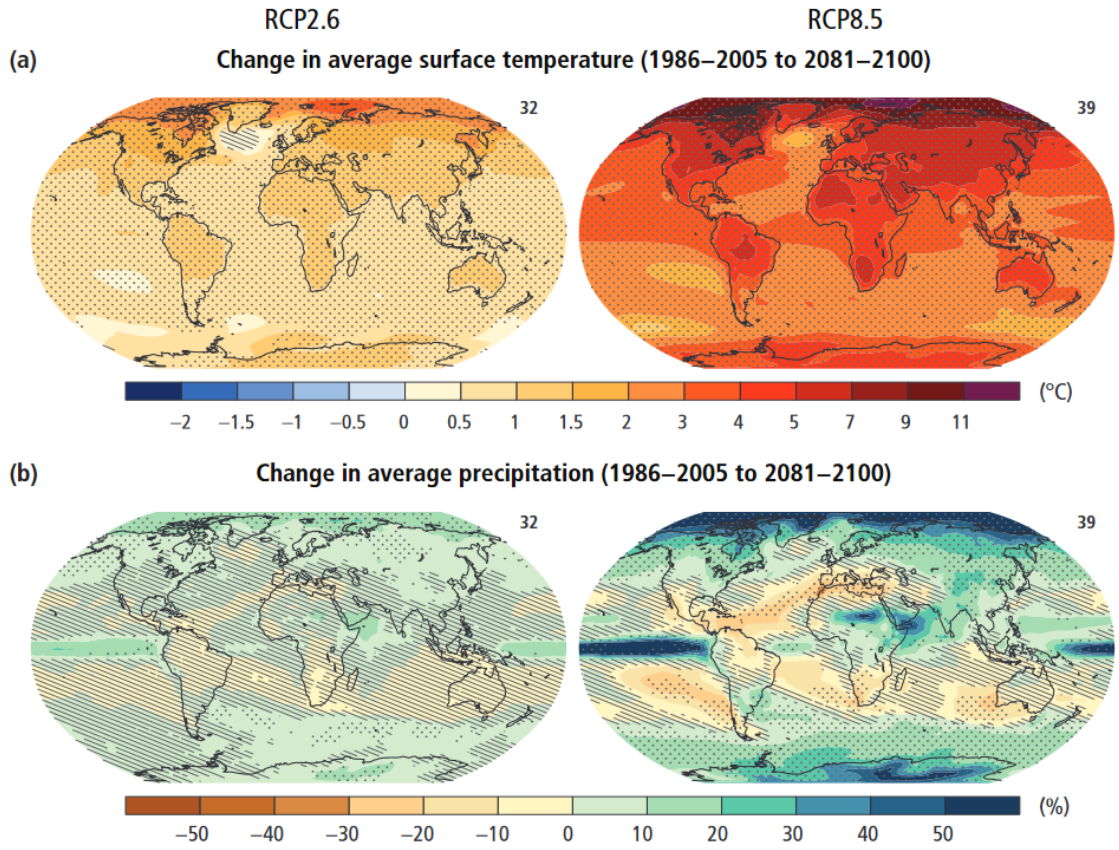

Figure A.1: Predicted global changes in average surface temperature and precipitation. The left globe panels present the model results of a stringent mitigation scenario (RCP2.6) that aims to keep global warming below 2°C above the pre-industrial average temperatures. The right globe panels present the model results from the more extreme scenario (RCP8.5, in which no additional efforts are made to constrain emissions (IPCC 2014). The number of models used to calculate the multi-model mean is indicated in the upper right corner of each globe. The dots show regions where the projected change is large compared to the naturally variability, while the diagonal lines show regions where the projected change is less than one standard deviation of the natural variability. This figure is from IPCC 2014

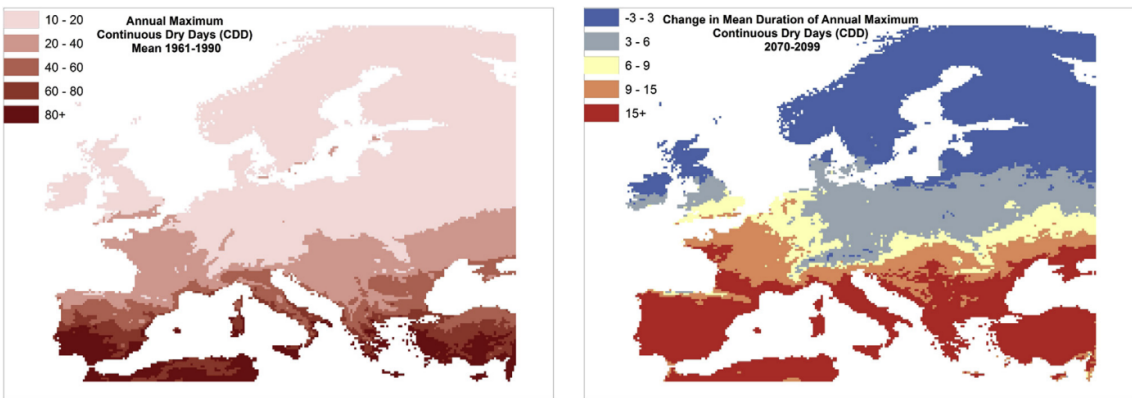

Figure A.2: Current patterns and future predictions for the longest period of the number of continuous dry days in Europe. This figure is from Lindner et al. 2014.

A.1.2 Sustainable forest management practices

Forests are increasingly impacted by climate change, such as an increase in wildfires, pests, windstorms, and drought. This then has further consequences on forest ecosystem services, such as carbon sequestration, biogeochemical cycling, and wood production (Millar and Stephenson 2015). Different strategies for sustainable forest management can induce short or long-term effects at the stand-level (Vilà-Cabrera et al. 2018). Two short-term strategies are the reduction of stand density and the management of the understory. Thinning removes trees to increase growth and stand health, and can reduce fire risk (Hurteau et al. 2008), as well as stimulate resistance to drought (D'Amato et al. 2013) and to pests (Waring and O'Hara 2005). Removing the understory cover further reduces fire risks (Adams 2013). Most studies involving the effect of management strategies focus on these two short-term strategies in regards to drought and fire disturbances (Vilà-Cabrera et al. 2018)).

Long-term strategies, such as increase tree diversity, are less common because results may not be immediately observed. However, these can be very advantageous for forests to acclimate to future disturbances. Promoting mixed species plantations can involve mixing different species or genotypes, which can improve resistance and recovery abilities to drought (de Andrés et al. 2017; Isbell et al. 2015; Pardos et al. 2021; Pretzsch et al. 2013b), pests (Guyot et al. 2019; Jactel et al. 2021), and fire (Hély et al. 2000; Jactel et al. 2017), by a variety of mechanisms such as niche partitioning or differing responses to stressors (del Río et al. 2017). This can also involve promoting forest structural diversity by having uneven aged forests, which are also expected to have a higher stability to disturbances (Martín-Alcón et al. 2010). Another long-term sustainable forest management strategy is changing species or genetic composition. This aims to change current species composition to have species or genotypes which are better adapted to the conditions predicted in future years. This can be done by assisted migration, in which species in the northern hemisphere for example, are moved either from their southern geographical range or completely replaced by species located further south, which have similar climates in the present to those expected in the future in the northern areas (Vitt et al. 2010). **While various strategies exist, in this thesis, the work will focus on the impact of promoting mixed tree species on forest functioning**.

However, there are trade-offs that need to be recognized when implementing a management strategy. There can be indirect effects of a particular choice of management strategy, which may maximize forest resilience but reduce another ecosystem function. For example, thinning has been found to positive influence drought resistance (Sohn et al. 2016), but may decrease decomposition rates (Bravo-Oviedo et al. 2017). Important trade-offs can exist among different land-management objectives, and tools have been created to help calculate their costs and benefits (Bradford and D'Amato 2012).

A.2 Forest functioning

Forests are major terrestrial ecosystems that are important culturally, economically, ecologically and socially, providing a multitude of ecosystem functions and services (Gamfeldt et al. 2013; Ratcliffe et al. 2017). Namely, a few important services on a global scale are wood production, carbon (C) storage, and nutrient cycling. Prior to diving into the main subject of this thesis (nutrient cycling), a brief overview of tree functioning is presented below.

Trees are characterized by their hard, woody stems (trunk), which has a role for the tree's structure and transport of water and nutrients. The trunk is made of the bark, with an outer periderm for protection and inner secondary phloem for the conduction of phloem sap (Pallardy 2008). This is the descending sap made of water and sugars synthesized by the leaves. The vascular cambium separates the bark from the secondary xylem; this compartment is made up of sapwood, the cells which transport the ascending sap made of water and mineral ions from the roots, and of heartwood, the non-conducting cells at the center of the tree which have a structural function (Pallardy 2008). The water transport from the soil to the atmosphere *via* the plant happens in a soil-plant-air continuum, which is connected *via* liquid water (Fig. A.3). The very low concentration of water vapor in the air, or water potential, around -100 MPa compared to the pressure within the plant leaf, drives the water loss from leaves, and thus the movement of water up the xylem, i.e. the water potential gradient (Kirkham 2014). This water loss, or evapotranspiration, is controlled by the stomata in the plant leaves (Klein 2014).

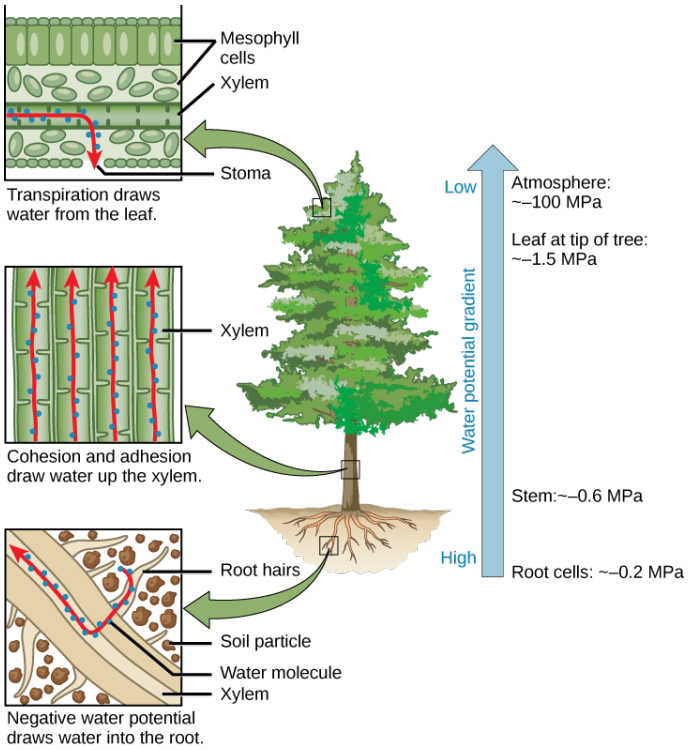

Figure A.3: Diagram depicting the movement of water in trees. The water goes from high water potential in the soil, to low water potential in the atmosphere. Ref: OpenStax Biology.

A.2.1 Tree growth strategies

In the spring, as the weather starts to warm and soil activity resumes, trees start the process of new leaf growth by remobilization of internal nutrient reserves. The evergreen foliage (needles or leaves) remain year-round and allow for year-round photosynthetic activity, as long as the temperatures are mild enough. This kind of foliage has developed to protect their leaves with a waxy coating, and having a small leaf surface area, to prevent water loss and survive other disturbance such as frosts. Deciduous leaves invest their resources into having more leaf surface area to maximize photosynthesis, since their leaves are out for only part of the year.

Another distinction between species types is the reproductive strategy: angiosperms, which have flowers and rely on pollination for fertilization, and gymnosperms, which have cone-bearing fruit, relying predominantly on the wind for fertilization, although some angiosperms also rely on wind for fertilization. Leaf type also distinguishes species, with broadleaves having flat leaves and conifers having needles. While many deciduous species are broadleaves and angiosperms and evergreen species are conifers and gymnosperms, there do exist exceptions. Larch, for example, is a deciduous conifer, a gymnosperm which loses its needles in the fall. There are also a handful of deciduous trees which keep their leaves past autumn, such as the Marcescent species (i.e. beech and oak), but this mainly occurs in young trees of these species and these leaves are dead. Coniferous

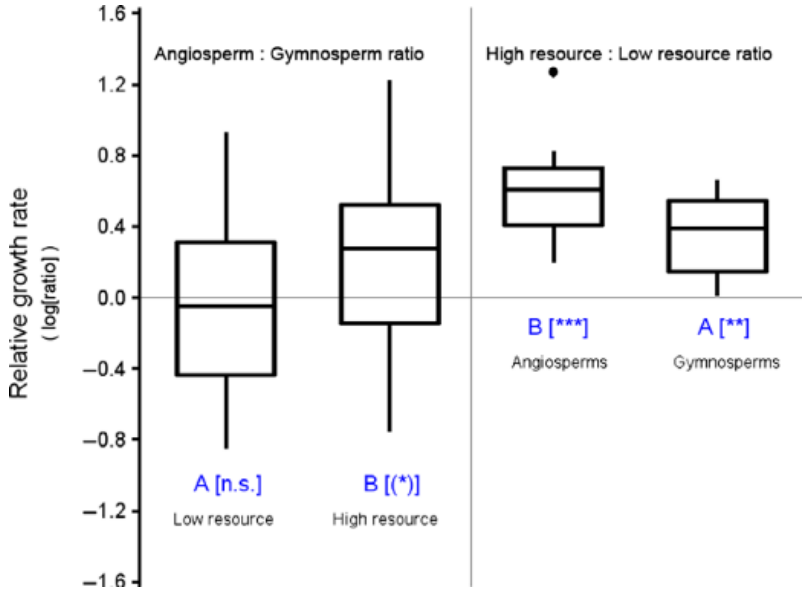

Figure A.4: Growth rates of angiosperm *vs.* gymnosperm tree species under low and high soil resource. Figure from Augusto et al. 2014.

species are resource conservative, having evolved in nutrient poor and xeric ecosystems. They are consequently characterized by higher nutrient use efficiency and lower litter nutrient quality than deciduous species (Augusto et al. 2014; Berendse and Scheffer 2009; Chapin et al. 1993; Pastor et al. 1984). When resources are scarce (i.e. nutrients and/or water), angiosperms can have similar growth rates to gymnosperms (Fig. A.4), while they are more competitive in habitats with high resources (Augusto et al. 2014). In general, conifers, which are tracheid bearing species, are on average two fold more resistant to drought stress (cavitation of xylem water conduits) than angiosperms. Additionally, they are on average more resistant to frosts by having smaller xylem conduit diameters than angiosperms, the vessel bearing species (Augusto et al. 2014).

As for differences in nutrient cycling between the different species types, angiosperms induce larger nutrient fluxes than the coniferous gymnosperms. Angiosperms usually have leaves with a higher nutrient content, which leads to a high flux of nutrients returning to the soil *via* the litterfall, and demand of nutrients from trees for growth. The differences in physiology and nutrient demands from these species with different growth strategies are important to keep in mind when studying the effect of mixing tree species (Biodiversity Effects - Section A.3). **In this thesis, the combination of deciduous and evergreen species will be studied when looking into the effects of promoting mixed species on forest functioning**.

A.2.2 Nutrient cycling in forests

The nutrients across terrestrial ecosystems which mainly constrain growth and activity of living organisms (including plants and microorganisms) are nitrogen [N] and phosphorus [P] (Elser et al. 2007). Plants require N in largest quantity (after carbon), and it comprises 1-5% of the plant dry matter. N plays a central role in plant metabolism and growth: within the plant, it makes up proteins, nucleic acids, chlorophyll, co-enzymes, phytohormones and secondary metabolites. P, although present in lower quantity, is also a key element for plant functioning. It is a structural element in nucleic acids and plays a key role in energy transfer and for transfer of carbohydrates in leaf cells. Both of these nutrients are often considered limiting for plant growth (Hou et al. 2021; LeBauer and Treseder 2008). Nitrogen can enter the soil *via* atmospheric biological N_2 fixation by certain plants with nodules, which form a symbiotic relationship with the rhizobia bacteria. Nitrogen, and to a less extent P, can also enter the soil *via* atmospheric deposition. However, P predominantly enters the soil *via* bedrock dissolution, although in some cases N can also enter by the weathering of soil rocks (Houlton et al. 2018). 'Young' soils tend to be enriched in P as both the physical and chemical alteration of the bedrock enriches the soils in P. Over time, soils decrease their P concentration due to its slow renewal unable to offset the losses due to uptake and leaching. Because nitrogen fixation can increase soil N availability, N tends to accumulate over geological timescales. Thus, over time, soils evolve from having high P and low N availability, to older soils which are richer in N and poorer in P (Walker and Syers 1976; Wardle et al. 2004).

On a smaller timescale, N and P are also cycled at both a global and ecosystem scale. In forests, nutrients are released from leaf and root litter and follow subsequent transformations to become available for nutrient uptake and use by all organisms in the ecosystem, including both trees and soil microorganisms. These different steps play an important role in controlling nutrient cycling in terrestrial ecosystems (Achat et al. 2013b; Mooshammer et al. 2012; Prescott 2010). While other elements (i.e. potassium, calcium, manganese, magnesium, sodium, zinc, iron, etc.) are also essential for plant nutrition (Marschner 2012), **the work of this thesis will primarily focus on nitrogen and phosphorus cycling**.

A.2.2.1 Nitrogen cycle

In general, N can enter the soil system from root and leaf litter, animal excrement, microbial turnover or by microbial N_2 fixation. Among the main mechanisms involved in soil organic matter decomposition, one process is based on extracellular enzymes (Sinsabaugh et al. 2002). Soil microorganisms release extracellular enzymes to acquire energy and resources by degrading neighboring soil organic matter (Burns 1982; Burns et al. 2013). Nitrogen, more specifically, is present in polymeric high-molecular weight (HMW) molecules that

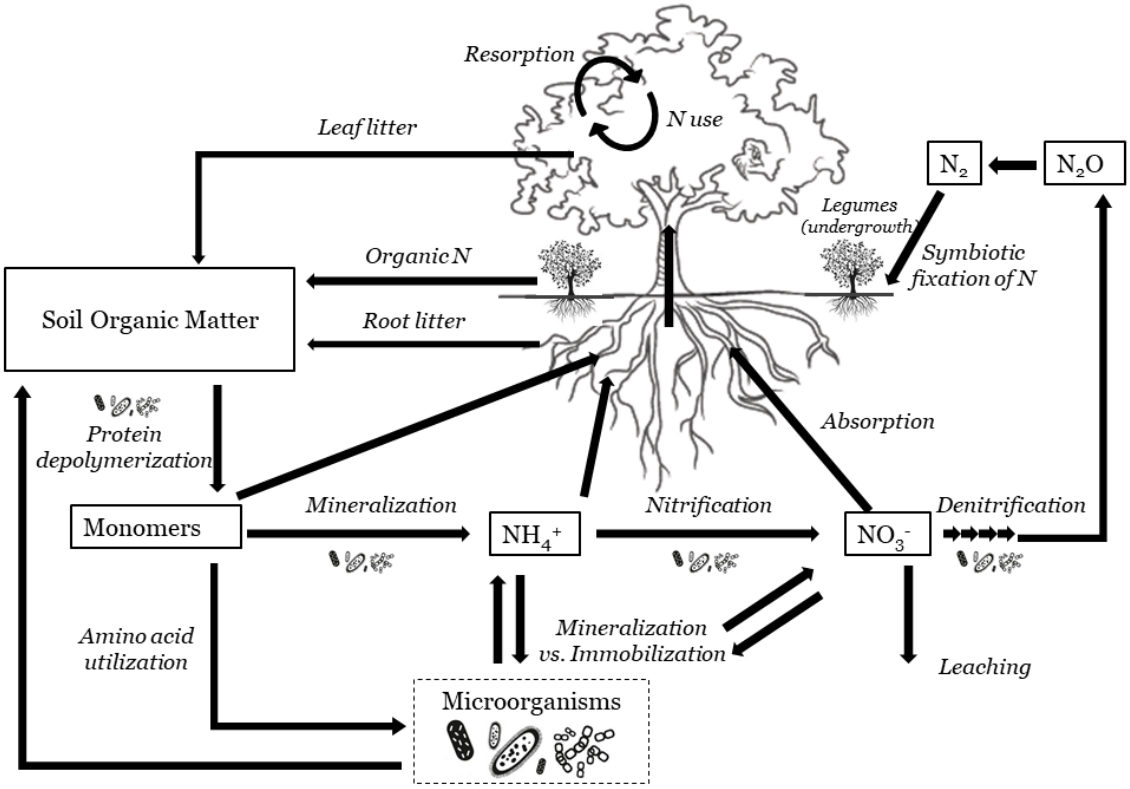

Figure A.5: An overview of the soil nitrogen cycle in forests. N_2O can also be produced from NH_4^+ and the organic N pool by autotrophic and heterotrophic nitrifiers. Adapted from Schimel and Bennett 2004.

need to be broken down (depolymerized) to smaller oligomers or monomers to become available to plants and microorganisms. This low-molecular weight (LMW) organic nitrogen can then follow one of several fates (Fig. A.5). Microbes can decompose this LMW organic N, such as amino acids, and mineralize it to ammonium ($NH_4{}^+$). Ammonium can subsequently be oxidized *via* nitrite (NO_2^-) to nitrate (NO_3^-) by nitrifying bacteria and archaea. Both ammonium and nitrate are ions readily taken up by plants and microbes. The preferred form of nitrogen uptake depends on plant-microbe competition (Kaye and Hart 1997), soil pH (Maathuis 2009), plant productivity (Nordin et al. 2001), as well as on neighboring plant identity and interspecific competition (Miller et al. 2007).

The mechanisms driving the inorganic nitrogen cycle in soils are reasonably well documented (Murphy et al. 2003), but processes regarding soil organic nitrogen breakdown prior to nitrogen mineralization are not as well understood. More recent evidence has shown that this depolymerization of HMW organic N (i.e. proteins, peptidoglycan and chitin) is the bottleneck of the soil N cycle (Hu et al. 2020; Schimel and Bennett 2004; Wanek et al. 2010). Organic nitrogen can also be directly taken up by plants and microbes without passing the nitrogen mineralization step (Neff et al. 2003). This direct capture of LMW organic nitrogen (i.e. monomers) by plants is dependent on both the presence

of a significant flux of LMW dissolved organic nitrogen and weak competition by soil microorganisms (Owen and Jones 2001). In boreal forests, the uptake rate of free amino acids (organic nitrogen compounds) can even exceed that of inorganic nitrogen uptake (Lipson and Näsholm 2001). In low fertility sites of temperate forests, the pool size of amino acids can be greater than that of inorganic nitrogen (Rothstein 2009). It has also been demonstrated that plants can take up intact proteins directly without the help of other organisms (Paungfoo-Lonhienne et al. 2008).

Once taken up by trees, nitrogen is transported up the xylem to different tree compartments, and especially to the leaves in order to fuel photosynthesis and leaf growth. At leaf yellowing, nutrients are redistributed from the leaves into the different tree organs, and stored especially in the xylem. This mechanism is known as retranslocation or resorption, which is the case for around 60% of N and P (Achat et al. 2018; Vergutz et al. 2012). Rates of N uptake, N use efficiency (the amount of biomass produced per unit of N taken up), and N resorption efficiency can differ strongly between species, available nutrients, soil types, and climates (Pastor et al. 1984; Vergutz et al. 2012; Vitousek 1982).

A substantial proportion of organic nitrogen escapes both mineralization and uptake, and partitions into the refractory soil organic nitrogen pool through microbial turnover and necromass stabilization, which is a rather stable pool with turnover times from dozens to thousands of years (Knicker 2011; Perakis and Hedin 2002). Finally, nitrogen can be lost from the soil system by leaching (drainage by percolating water) and gaseous emissions of NH_3 and NO, along with N_2O and N_2, the latter being byproducts of denitrification (Bai and Houlton 2009).

A.2.2.2 Phosphorus cycle

The main difference between N and P cycling in forests is that most of the pools of P in soils are not easily soluble, e.g. strongly adsorbed on oxide surfaces or unavailable in crystalized minerals, especially in bedrock. Thus, total soluble P in soils strongly depends on the state of ecosystem development with the desorption of P from mineral surfaces (clays, Fe, and Al oxides, carbonates), weathering from primary minerals, and dissolution from secondary compounds (CaP, FeP, MnP, AlP) (Fig. A.6). Similar to the N cycle, the mineralization of soil organic matter by microorganisms produces soluble phosphate ions, which are mostly immobilized by the microorganisms, taken up by trees, or leached. The decomposition and mineralization of soil organic matter is the largest P flux and highest biotic P turnover in forest ecosystems (Achat et al. 2013b). Phosphorus, like N, is resorbed and stocked in the tree's storage compartments prior to leaf senescence to minimize P losses. Precipitation of soluble P in soils can lead to the formation of secondary compounds, and adsorption of soluble P can bind P to mineral surfaces in the soil. The

phosphorus cycle in temperate forests is further detailed in the work of Sohrt et al. 2017.

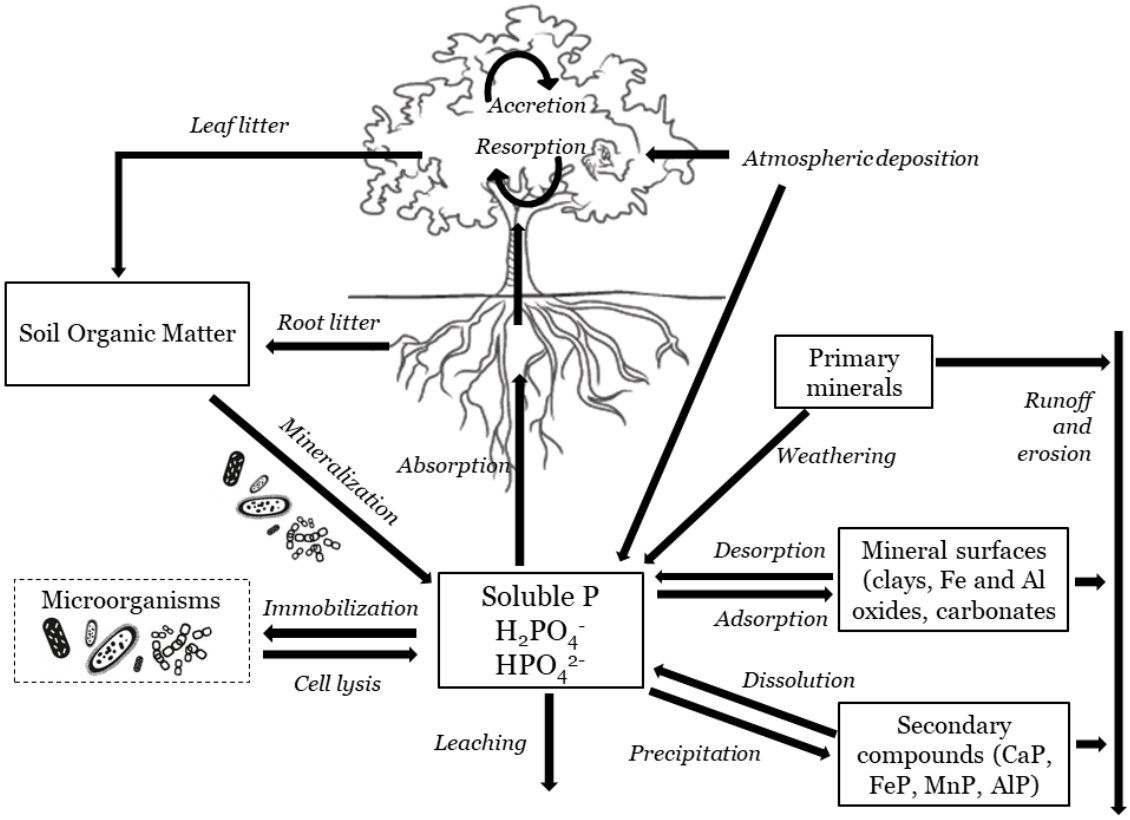

Figure A.6: An overview of the phosphorus cycle in forests. Adapted from Sohrt et al. 2017.

A.2.3 Plant-soil interactions

There are several plant-soil interactions which make up ecosystems as we know them. As previously mentioned, plant litter is an important input to soil organic matter. The leaf chemistry, its nutrient concentration, the amount of cellulose, lignin or tannin, its pH, and its surface area, can all affect the presence and composition of the decomposing soil fauna and microorganisms (Aerts 1997; Bray et al. 2012). Root litter, along with and microbial death and lysis, are also important inputs to the soil organic matter (Kögel-Knabner 2002; Liang et al. 2019). Many studies have shown a direct link between leaf and/or root litter on the associated microbial communities and biomass (Bani et al. 2018; Purahong et al. 2016b; Romaní et al. 2006). These soil microorganisms can then influence the plants in several ways. In a direct pathway, soil microorganisms may have a synergistic, symbiotic or antagonistic relationship with plants. For example, mycorrhizae are synergistic fungi with plants: plants provide fungi with needed C to grow, while the fungi help provide water and nutrients to the plants. However, there are pathogenic fungi, whose presence may harm or kill the plant. An indirect pathway with which soil microorganisms interact with plants is the way decomposers and organic nutrient mineralizers determine the nutrients

available in the soil, and thus the quantity of nutrients available for uptake by the plants. **The plant-soil interactions are key to understanding how tree diversity may influence belowground nutrient cycling**.

Plant-soil feedbacks (PSFs) are also increasingly impacted by global changes (Pugnaire et al. 2019). Globally, drought tends to decrease the quality of leaf litter, as carbon-based recalcitrant compounds are produced and nutrient resorption prior to leaf senescence is elevated under drought (Suseela et al. 2015). A forest impacted by drought may favor slow growing species, with a low quality litter, favoring the presence of fungi in the soil (Fig. A.7). This then can lead to low nutrient availability, thereby enhancing the feedback and favoring slow-growing resource conservative species. In a more humid environment, fast-growing species with high quality litter are favored, leading to a higher presence of bacteria in the soil, further leading to higher nutrient availability (Fig. A.7). An increase in drought, in addition to expected increases in temperature, which indirectly cause lower soil moisture, may thus have important consequences on forest ecosystems.

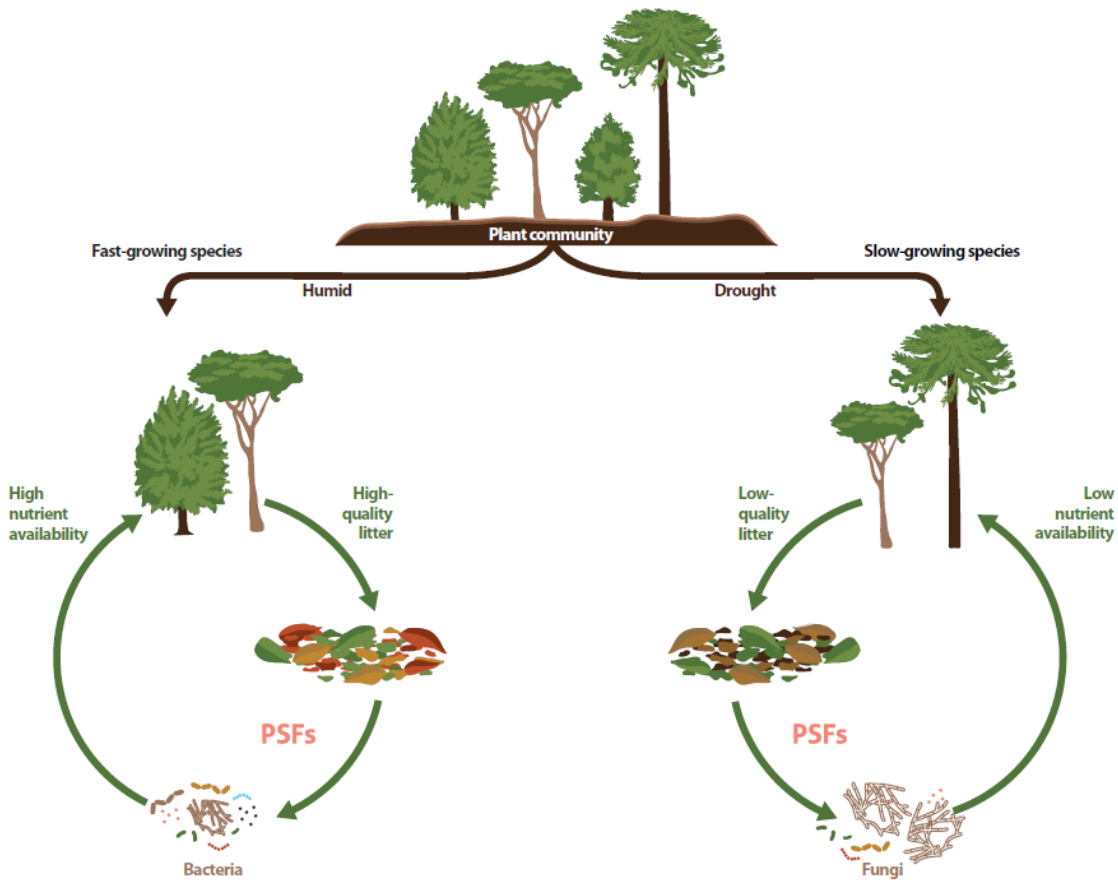

Figure A.7: Effects of drought on plant-soil interactions (PSFs). This figure is from Pugnaire et al. 2019.

A.3 Biodiversity effects

In order to investigate the impact of mixed species forests on nutrient cycling, a brief overview is given of the relationship between biodiversity and ecosystem functioning (Section A.3.1). The main results found for the impact of biodiversity on forests is presented (Section A.3.2), after which the impact of mixing tree species on various steps of nutrient cycling is detailed (Section A.3.3).

A.3.1 Biodiversity – ecosystem functioning relationships

Positive biodiversity – ecosystem functioning relationships were originally explained by two phenomena: complementarity effects or selection effects of species (Loreau et al. 2001). Species coexistence *via* niche differences or multivariate combinations of traits may explain complementarity effects, especially at smaller scales. Selection effects are due to competitive exclusion *via* fitness differences or single preference traits, such as plant height being a strong competition for light. In forests, this is true at larger scales with a dominant species (Cadotte 2017; Robinson et al. 2012). The impact of selection or complementary effects can be calculated and separated from one another by using an additive partitioning method (Loreau et al. 2001). Subsequently, these differences in biodiversity effects have also developed the "stress-gradient hypothesis", where plants with moderate stress are more likely to display facilitation interactions than those under low stress, where competition may take the upper hand (Michalet et al. 2006; Pretzsch et al. 2014). Indeed, stronger biodiversity-ecosystem functioning relationships have been found in drier climates, as water availability drives context-dependency for tree diversity studies (Ratcliffe et al. 2017).

The two mechanisms, complementary effects and selection effects, may also be explained in terms of productivity, with selection being driven by stochastic assembly in productive communities, and niche differences driving deterministic assembly in less-productive communities (Mori 2018). Additionally, the mechanisms underlying biodiversity effects may be explained using plant traits. Plants with conservative resource-use strategies may have higher biodiversity effects under high competition intensity, whereas plants with acquisitive traits may have strongest effects under low competition intensity (Fanin et al. 2018; Fichtner et al. 2017). Similarly, the mechanisms behind complementarity and selection effects may be explained by a community's functional diversity and functional identity, respectively (Tobner et al. 2016). In forests, for example, a gradient of functional diversity, from deciduous to evergreen trees, may test complementary effects independently of a gradient in species richness, which may be measured by community weighted mean of plant traits, a proxy for functional identity.

A.3.2 Effects of biodiversity in forests

In forests, mixed species plots may demonstrate higher productivity than monocultures due to an increase in structural heterogeneity (Pretzsch et al. 2016), or due to spatio-temporal complementarity, such as during light interception (Pretzsch et al. 2014). This is due to the large range of shade tolerance present in different tree species (Cordonnier et al. 2018a), which may be why selection effects are stronger in forests than in grasslands (Tobner et al. 2016). On average, mixed forests may provide more forest ecosystem services, revealing complementarity effects which produce positive biodiversity effects (Gamfeldt et al. 2013). Positive, synergistic effects occur when the mixture produces more (or has a faster rate) than the corresponding monoculture productivities (additive effect) (Fig. A.8). In terms of biomass production, this synergistic effect is known as "overyielding".

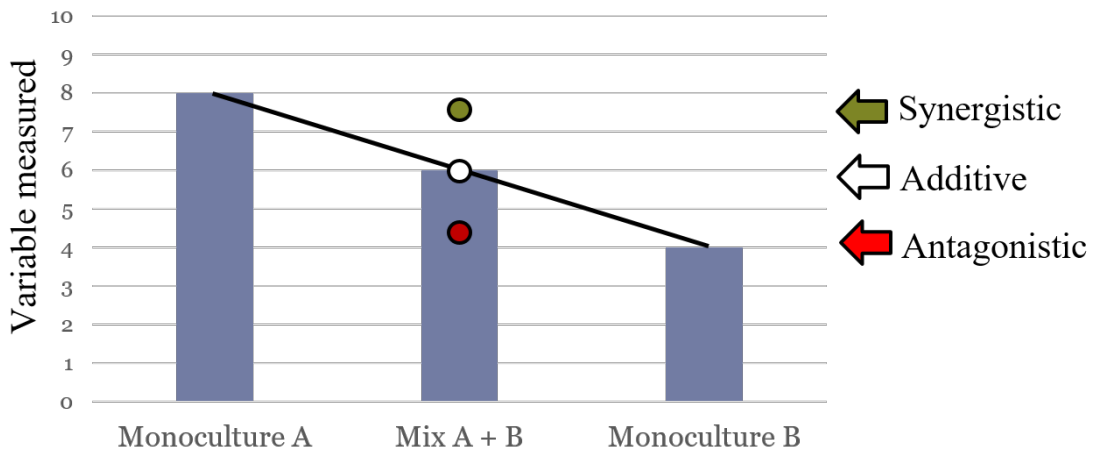

Figure A.8: Varying effects which biodiversity may have on a variable measured (i.e. productivity). An additive effect occurs when the mixture of a species A and B produced is the addition of the equivalent of half of each of the monocultures' production. Visually, it occurs at the midpoint when a straight line is drawn between the full production of both monocultures. An antagonistic biodiversity effect occurs when less than the addition of the parts of the monocultures is produced in the mixture, while a synergistic biodiversity effect occurs when the mixture produces more than this additive effect.

Globally, mixed species forests have been found to have a more stable production than pure stands over time due to overyielding, species asynchrony and favorable species interactions (Jucker et al. 2014a; Morin et al. 2014). Preserving or increasing tree diversity to maintain commercial wood productivity has also been giving an economic value ranging from $166 billion to $490 billion per year (Liang et al. 2016).

A.3.3 Effects of biodiversity on nutrient cycling

The effect of species richness and functional diversity on various parts of nutrient cycles have been previously investigated (Fig. A.5 & Fig. A.6). Litter decomposition is commonly

studied to determine how varying leaf litters can influence nitrogen inputs into the soil system (Hättenschwiler et al. 2005), as litter decomposition is one of the essential nutrient cycling processes that controls the availability of nutrients for plants and microorganisms. Leaves from different tree species can differ by their nutrient content, cellulose, lignin and tannin concentration, along with leaf surface area and pH. The effect of mixing various litters on the rate of litter mass loss, litter nutrient content, and decomposer abundance and activity vary greatly between different studies (Gartner and Cardon 2004). The effects have been found to be positive (Handa et al. 2014; Jewell et al. 2017), negative (Prescott et al. 2000), neutral (Sohng et al. 2014), or varying depending on the species' identities (Nurlaila Setiawan et al. 2016). A positive correlation has also been found between tree species richness and decomposition of cellulose, a common substrate in leaf litters (Joly et al. 2017).

An important part of litter decomposition involves the breakdown of organic matter by extracellular enzymes (Sinsabaugh et al. 2002). These are released by microorganisms to acquire energy and resources by degrading the neighboring soil organic matter (Burns 1982; Burns et al. 2013). Studying soil extracellular enzyme activities (EEAs) can thus give information on potential belowground microbial mineralization processes and how microbial communities drive C, N, and P cycling in soils (Allison et al. 2007; Fanin et al. 2016b; Henry 2013). Tree species can influence soil EEAs due to their varying litter qualities (Weand et al. 2010), and mixing tree species has been found to lead to an increase in soil EEAs (Alberti et al. 2017; Hacker et al. 2015).

Once organic matter has been broken down, it can be mineralized into various forms of easily-absorbed N ions (ammonium, NH_4^+ and nitrate, NO_3^-) for uptake by trees and immobilization by microorganisms (Fig. A.5). Net N mineralization is expected to be higher in deciduous forests than in coniferous forests (Côté et al. 2000; Gosz 1981; Melillo 1981), due to the nutrient-rich litter stimulating belowground microbial activity, although other factors such as the indirect effect on soil properties or understory communities can also play a role affecting mineralization (Augusto et al. 2015; Brierley et al. 2001; Lejon et al. 2005). Mixing broad-leaved with needle litters is the mixture that has most often been found to increase N mineralization beyond predicted values (Chapman et al. 1988; Fyles and Fyles 2011; Gartner and Cardon 2004; McTiernan et al. 1997; Salamanca et al. 1998; Williams and Alexander 1991), as a species with nutrient-rich litter may enhance the decomposition and nutrient release from species with low-quality litter when mixed together (Chapman et al. 1988; Finzi and Canham 1998; Wardle et al. 1997). This may be due to a change in microbial community composition (Chapman et al. 2013), leading to synergistic effects of mixing on litter decomposition and subsequent mineralization. This could also be due to an increase in microbial biomass and activity, as increasing tree species diversity has been found to do so (Chen et al. 2019a; Lucas-Borja et al. 2012;

Thakur et al. 2015). Although less frequent, there have also been varying responses of belowground processes to species mixtures (Dijkstra et al. 2009). Overall, an increase in litter decomposition, soil EEAs, and mineralization may lead to an increase in soil nutrient availability in mixed species forests (Richards et al. 2010).

Mixed plantations have also led to increased root stratification (Rothe and Binkley 2001) and fine root productivity (Brassard et al. 2013; Ma and Chen 2016), although this is not always the case (Archambault et al. 2019; Bolte and Villanueva 2006; Finér et al. 2017). Root stratification may occur in mixtures, meaning that the root systems of different species may be vertically separated with depth along the soil profile. Spatial complementarity in forests may occur, due to niche partitioning along the soil profile (Brassard et al. 2013) or depth-specific asymmetric competition (Altinalmazis-Kondylis et al. 2020). This spatial complementarity, along with a potential increase in root biomass, may optimize nutrient acquisition in mixed forests. Nutrient uptake may also be increased in mixed forests by a plasticity in uptake of different forms of N, which reduces the competition between neighboring species (Millar et al. 2007). Facilitation mechanisms between associated species in a mixture, such as hydraulic lift (Zapater et al. 2011), where a deeper rooting species 'brings up' water to shallower soil layers, may be especially advantageous in mixed stands during drought events.

Finally, mixed plantations have been found to have more efficient aboveground nitrogen use (Schwarz et al. 2014), which may be due to changes in nutrient residence times in the plant biomass (Binkley et al. 2004). However, nutrient use efficiency (NutUE) may also decrease in mixtures, due to the interaction between different limiting resources (light, nutrients, water) and their efficiency of use (Richards et al. 2010). Because NutUE is determined by the annual aboveground net primary productivity (ANPP), annual litterfall mass, and litter nutrient concentrations, various effects of tree diversity on these different parameters may lead to changes in NutUE with mixing. Past studies frequently found a positive effect of species richness on production of litterfall (Huang et al. 2017; Scherer-Lorenzen et al. 2007) and aboveground productivity (Chisholm et al. 2013; Liang et al. 2016; Morin et al. 2011; Piotto 2008; Toïgo et al. 2015; Zhang et al. 2012). This effect was attributed to an increase in structural heterogeneity (Pretzsch et al. 2016), or spatiotemporal complementarity such as during light interception (Pretzsch et al. 2014; Williams et al. 2017), caused by a range of shade tolerance of different species (Cordonnier et al. 2018a). NutUE is also partially influenced by nutrient resorption efficiency (NutRE) (Hayes et al. 2014), i.e., the ability for trees to efficiently retranslocate nutrients prior to litterfall, notably because the calculation of NutUE depends on the quantity of remaining nutrients in the litter. Prior to leaf abscission and to a lesser degree, throughout a leaf's life, nutrients are remobilized from the leaves into developing tissues or stored for later use (Wright and Westoby 2003). When there is high nutrient availability in soils (i.e. possibly in mixed

species forests (Richards et al. 2010) or with higher water supply), there is less overall cost for the tree to take up nutrients in soil. Thus, fewer nutrients need to be remobilized from the green leaves (Achat et al. 2018; Wright and Westoby 2003), resulting in increased litter nutrient concentrations, which can lead to decreased NutUE with mixing (Hayes et al. 2014).

Although there are varying responses to mixed species for the different steps of nutrient cycling, this elucidates a strong potential for an effect of tree biodiversity on nutrient cycling. These experiments, both in manipulated plantations and in natural forests, have investigated the effect of species richness and functional diversity on these different aspects of nutrient cycling, but few have studied nutrient availability, uptake, and use efficiency concomitantly (Richards et al. 2010).

A.4 Water availability effects

In this section of the general context, the effects of water availability in forests is presented (Section A.4.1), after which previous studies regarding the impact of reduced water availability on the various steps of nutrient cycling are presented (Section A.4.2). In order to be consistent and clear throughout the thesis (Slette et al. 2019), we define 'low' or 'reduced' water availability as an umbrella term used to represent prolonged reduction in precipitation, leading to low soil moisture and water availability.

A.4.1 Effects of water availability in forests

In general, forests may be affected by reduced water availability directly, such as during droughts, by reducing leaf conductance and water uptake, and potentially losing leaves early (Schlesinger et al. 2016). This may then affect the input of leaf nutrients, which in turn affects the decomposers and microorganism activity in soils. Reduced water availability may also indirectly affect forests by increasing herbivory, because the trees have fewer resources to allocate to protective compounds, such as terpenes, during droughts (Jactel et al. 2017). When leaves are attacked and defoliated, there are fewer nutrients that can be retranslocated into the different compartments of the tree prior to leaf senescence. Disturbances can be so damaging that the C cycle can be severely affected, such as reducing net ecosystem annual CO_2 exchange by 41-55% in upland forests after a defoliation (Clark et al. 2010).

The reduced stomatal conductance causes a reduction in photosynthesis and transpiration which causes a reduction in shoot biomass (He and Dijkstra 2014). In a deciduous broadleaf

forest in the USA, the annual accumulation of woody biomass decreased by 41% following drought stress, which had reduced the number of days of wood production by 42 days in a year (Brzostek et al. 2014). Responding to drought, trees are more likely to grow deeper roots or to translocate their C to subsurface roots to improve water and nutrient uptake. However, previous studies have found varying results on the impact of reduced water availability, deducing that these effects depend on the species, soil type, and on the specific nutrients. One of the ways that trees may reduce the water stress is by one of two types of mycorrhizal associations: ectomycorrhizal (ECM) fungi, which do not penetrate the root cells and arbuscular mycorrhizal (AM) fungi, whose hyphae penetrate the root cell walls. Trees with ECM associations are more tolerant to drought stress then trees with AM networking, which may be due to their functional differences and the host trees and fungi's belowground C allocation (Brzostek et al. 2014).

A.4.2 Effects of water availability on nutrient cycling

Water is necessary for all microorganism activity in soils. Prolonged drought causes a decrease in soil moisture, which subsequently causes a decrease in soil microorganism activity in aerobic conditions (Kreuzwieser and Gessler 2010). Indeed, soil moisture is a major driver of soil microbial activity (Brockett et al. 2012). In forests, reduced water availability may lead to a decrease in rates of litter decomposition (Zheng et al. 2017), enzyme activities (Sardans and Peñuelas 2005), nitrification (Chen et al. 2011), and mineralization (Borken and Matzner 2009). A recent global meta-analysis concluded that increased precipitation significantly increased N-related enzyme activities (Xiao et al. 2018). However, the effects of water availability may vary with soil depth due to the dynamics of water runoff and infiltration from the topsoil to deeper soil. Increased precipitation is thus expected to have a stronger positive influence on belowground processes in the top soil, where soil moisture increases most with precipitation. Enzymes in deeper soil, which have less varying moisture, may thus be less affected by changes in precipitation (Fanin et al. 2020).

This decrease in process rates with a decrease in water availability, can subsequently lead to a decrease in soil available nutrients (Augusto et al. 2017; Bengtson et al. 2005). The negative effects of drought on microbial processes can be compensated by an increase in rates after rainfall (He and Dijkstra 2014). Over time, trees may adapt to prolonged reduced water availability. However, it is more likely that there will a drought legacy effect on nutrient cycling for several years after an intense drought (Gessler et al. 2017).

A decrease in water may also cause a decrease in nutrient uptake because nutrients are mobilized by water in soils and into trees. This diffusion and mass flow is required to move nutrients from the soil into the root system (Marschner 2012). Once nutrients have entered

the root, they are distributed within the tree in channels within the xylem and up into the leaves. A decrease in soil water content thus causes a decrease in nutrients reaching the leaves. This is particularly the case of nutrients that predominately enter roots through convection, such as calcium or magnesium (Augusto et al. 2011). Generally, trees have been found to reduce ANPP under drought conditions, and reduce allocation to litterfall (Brando et al. 2008). A tree's resorption efficiency can be affected by changes in soil moisture (Achat et al. 2018; Killingbeck 1996). Another entrance of nutrients into the soil system is *via* throughfall and stemflow, when rainfall induces leaching of mobile nutrients such as potassium from leaves and stems. A decrease in rain could then decrease the rain flow of these nutrients into the soil, reducing nutrient cycling.

It is important to note that there exists a hierarchy between water and nutrient acquisition. Water availability is a limiting resource for the rate of photosynthesis, so it may be argued that it also affects the rate at which trees accumulate carbon per unit of nitrogen (the rate of biomass increase) (Knops et al. 1997). If there is not enough water in the soil system (i.e. an intensive prolonged drought), roots will be extremely limited in their ability to take up nutrients from the soil, even if they are abundant (Kreuzwieser and Gessler 2010). Therefore, while drier soils can present slower microbial activity, the effect of increasing soil moisture may be constrained by soil nutrient availability (Cusack et al. 2019).

Several studies have looked at the effect of various levels of water availability or at the effect of drought on forest ecosystems in order to determine how both individual trees and forest stands respond to water stress. However, few studies have holistically examined water availability effects on nutrient cycling (i.e. on the important steps concomitantly), and even less is known about how the effects of reduced water availability will vary between monocultures and mixed forests.

A.5 Effect of the interaction between biodiversity and water availability

Because reduced water availability negatively affects forest ecosystem functions and services globally, it is necessary to examine strategies and adaptations that will reduce this effect. One of the ways to offset these negative effects may be to increase the number of different tree species into forests, which may increase its resilience or adaptive capability to drought, especially in drought-prone environments (Grossiord et al. 2014; Pretzsch et al. 2013a; Pretzsch et al. 2014). This may be explained by a niche partitioning due to changes in root stratification, allowing different species to use nutrients and water at different depths of the soil profile (Brassard et al. 2013; Richards et al. 2010). Many studies that investigated the interaction between tree biodiversity and drought have only studied their effect on aboveground indicators, such as total aboveground biomass, tree rings, height, and diameter (Kardol et al. 2018; Lebourgeois et al. 2013; Pretzsch et al. 2013a). This interaction has received less attention for belowground processes, which are crucial to understand nutrient cycling.

Not only are mixed forests an appropriate solution to drier climates, their biodiversity effect on ecosystem functioning may be even stronger in this context. In a meta-analysis of biodiversity experiments in European forests looking at nutrient processes, wood production, regeneration, and resistance, researchers found that there were stronger biodiversity-ecosystem functioning relationships in drier areas with more functionally diverse tree species (Ratcliffe et al. 2017). Hence, biodiversity could be even more important to mitigate future drier climates in forests. This study provides a good insight on this role for only a few processes of nutrient cycling, including litter decomposition, soil C/N ratio, microbial biomass, soil C stock, wood decomposition and nitrogen resorption efficiency. In a study linking various factors with microbial communities, Brockett et al. 2012 found that these parameters were closely associated with soil moisture and organic matter content, but that tree species composition also played a secondary role. Different functional traits among tree species may also drive soil microbial resistance and resilience to drought, by generating diverging soil biochemical properties (Rivest et al. 2015). However, these are only parts of nutrient cycling, and more notably, the studies that were used for the meta-analysis did not measure all of these steps together. In order to better understand how changes in water availability may impact tree diversity effects, there is a need to study this interaction for a majority of nutrient cycling steps.

There is also an important dependency of the environmental context when studying biodiversity-ecosystem functioning relationships (Adair et al. 2018; Forrester 2014; Jucker et al. 2014a; Jucker et al. 2014b). It may be even more important when studying the interaction between biodiversity and reduced water availability, because varying ecosystems

respond differently to both of these effects. For example, researchers have found that maximal tree biodiversity effects on ecosystem functioning occur when there is low soil fertility, resource heterogeneity causing resource partitioning, different root profiles, and favored species functional dissimilarity (Ratcliffe et al. 2017). Climate has also been found to modulate biodiversity effects on forest productivity (Jucker et al. 2016). Additionally, biodiversity effects are overall expected to be even greater in a drought scenario, where the growth rates are lower (del Rio et al. 2014).

A study that looks at the effects of the interaction between biodiversity and reduced water availability integrally on nutrient cycling by measuring the varying steps concomitantly in more than one context, would thus provide crucial insight to identify the ecological mechanisms by which biodiversity may allow forests to cope with drought events.

B OVERALL OBJECTIVES

In order to offset the negative effects of reduced water availability, several forest adaptation strategies have been introduced to maintain forest productivity, among which increasing the local diversity of tree species (species richness and functional diversity) is put forth as an efficient practice. While effects have been estimated by looking at natural forest ecosystems (Pretzsch et al. 2016) and modeling (Mora et al. 2014), studies which experimentally manipulate both tree biodiversity and water availability are few. There is a need to study how this interaction affects the different dimensions of ecosystem functioning, and particularly of nutrient cycling (Fig. B.1).

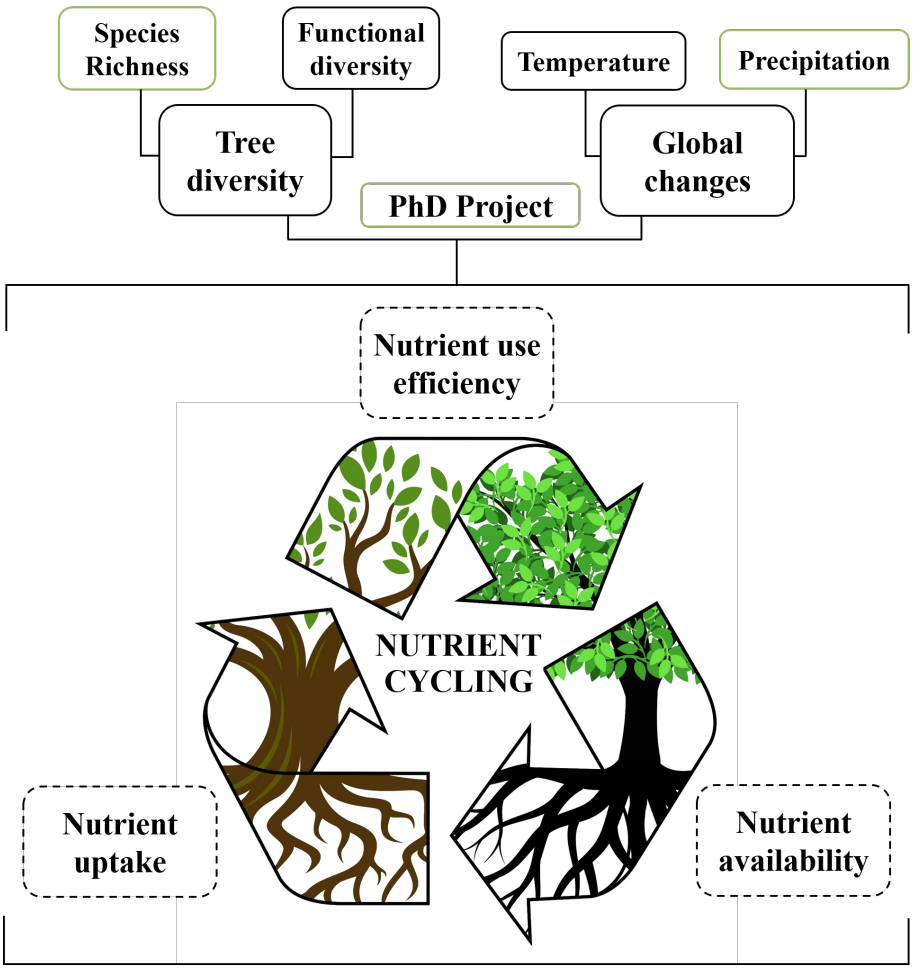

Figure B.1: Graphical representation of the thesis organization. All four chapters focus on the interactive effect of tree diversity and water availability on various steps of the nutrient cycle in forests. Chapter 1 focuses on potential spatial complementarity on nutrient availability, while chapter 2 focuses on soil organic nitrogen breakdown. Chapter 3 focuses on potential temporal complementarity on soil nutrient availability and uptake by trees. Finally, chapter 4 tests the whether mixed stands improve nutrient use efficiency, especially at low levels of water availability.

The purpose of this thesis was to determine whether changes in water availability may affect potential tree mixing effects (with a focus on a deciduous and evergreen species: birch and pine) on nutrient cycling (Fig. B.2). To do so, I used the following components of the Production Ecology Equation (Monteith 1977), as a basis to structure the different studies: soil nutrient availability, tree nutrient uptake, and nutrient use efficiency (Richards et al., 2010). This led to the development of four chapters for this thesis, in which I looked into the interactive effects of tree diversity and water availability: (1) spatial complementarity of soil nutrient availability, (2) soil organic N breakdown, (3) temporal complementarity of soil nutrient availability and nutrient uptake, and (4) nutrient use efficiency.

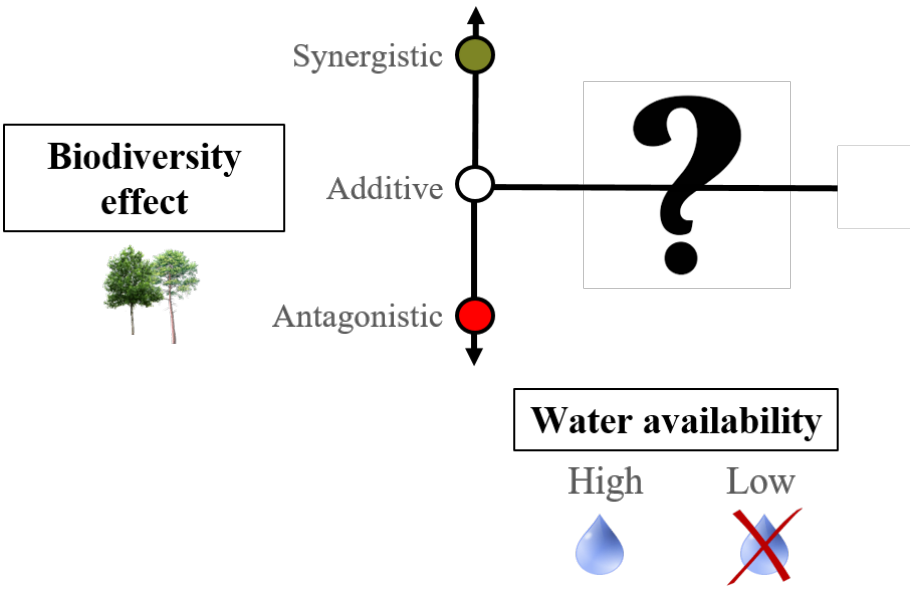

Figure B.2: A graphical representation of the overall Ph.D. objectives. The interaction between biodiversity and water availability on nutrient cycling remains elusive. Here, we refer to how low water availability may change the biodiversity effect (antagonistic, additive, or synergistic) on the different parts of nutrient cycling, when compared to optimal water resource conditions.

B.1 Hypotheses

Chapter 1: Spatial complementarity

Chapter 1 aims to study the interactive effect of tree mixing and water availability along the soil profile at five soil layers, to test potential spatial complementarity on available nutrients and soil extracellular enzyme activities (EEAs). Three sub-hypotheses were tested:

- ($H_{1.1}$) EEAs would be higher in the two-species birch-pine mixture than in the corresponding pure plots since mixing different litter qualities may lead to more synergistic litter decomposition (Chapman et al. 2013; Cornwell et al. 2008). Specifically, we hypothesized that the effect of mixing trees on EEAs should be higher in the topsoil layer, where the effect of tree species identity is highest (Augusto et al. 2002) and where the organic matter quantity is highest and richest in nutrients (Jobbágy and Jackson 2001; Melillo et al. 1989).
- ($H_{1.2}$) An increase in water supply *via* irrigation would have a positive effect on EEAs by alleviating the water constraint (Sardans and Peñuelas 2005; Xiao et al. 2018). We anticipated that the effect of increasing water availability should be highest in the topsoil, mainly because microbial communities at the soil surface are often more subjected to water stress than those living in deeper soil horizons (Fanin et al. 2020).
- ($H_{1.3}$) The positive effect of mixing two tree species on EEAs would be strongest in the plots experiencing the dry summers. This is because mixing tree species with contrasting root exploration strategies may favor drought resistance through facilitation mechanisms (Bello et al. 2019).

Hypothesis 1: Positive mixing effects on soil nutrient availability and enzyme activities should increase under low water availability (Fig. B.3).

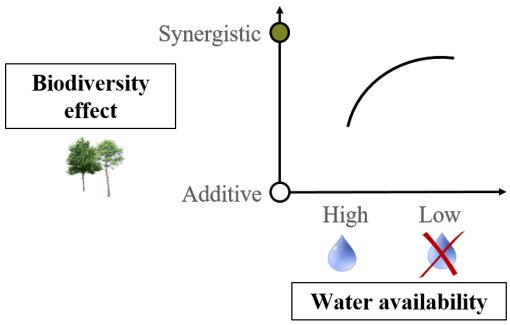

Figure B.3: Hypothesis for chapter 1.

Chapter 2: Soil organic nitrogen breakdown

Chapter 2 aims to dive further into the potential interactive effect of tree mixing and water availability on soil organic matter breakdown specific to nitrogen (protein depolymerization), using a novel high throughput isotope pool dilution method. Three sub-hypotheses were tested:

- ($H_{2.1}$) Mixing tree species would lead to an increase in gross protein depolymerization due to both a synergistic litter decomposition (Chapman et al. 2013; Cornwell et al. 2008) and an increase in microbial biomass (Chen et al. 2019a; Lucas-Borja et al. 2012; Thakur et al. 2015).
- ($H_{2.2}$) An increase in water availability would increase protein depolymerization due to its stimulation of microbial activity (Brockett et al. 2012).
- ($H_{2.3}$) Facilitation mechanisms between microorganisms to decompose litter with differing quality have been found to be stronger under limited water conditions (Ratcliffe et al. 2017), so we expected the positive effect of mixing to be strongest in the plots subject to the dry summers of southwestern France.

Hypothesis 2: Positive mixing effects on soil organic nitrogen processes should increase under low water availability (Fig. B.4).

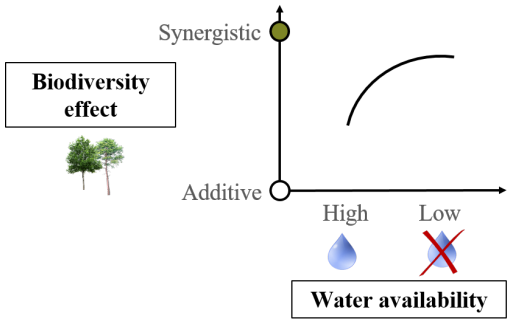

Figure B.4: Hypothesis for chapter 2.

Chapter 3: Temporal complementarity

Chapter 3 aims to study the interactive effect of tree mixing and water availability on various belowground processes to test potential temporal complementarity of soil nutrient availability and tree nutrient uptake in the mixture. More specifically, three sub-hypotheses were tested:

- ($H_{3.1}$) In line with $H_{1.1}$ and $H_{2.1}$, regarding an increase in decomposition with mixing varying leaf litters, mixing tree species would lead to an increase in soil organic matter mineralization (Borken and Matzner 2009) and subsequent higher soil nutrient availability in mixed stands compared to monocultures. More specifically, we expected the mixing effect on soil nutrient availability to be strongest during the growing period, due to higher microbial activity at this time (Gallardo and Schlesinger 1994; Kang et al. 2009).
- ($H_{3.2}$) Asynchrony in nutrient uptake between the evergreen conifer and broadleaf deciduous would be strongest at budburst, due to differences in phenology and growth strategies (Carnicer et al. 2013), leading to maximum uptake at this time.
- ($H_{3.3}$) Irrigation would lead to an overall positive effect on process rates, due to its favoring of microbial activity (Brockett et al. 2012). This should be strongest in the peak dry summer, when there would be the largest difference in soil moisture between treatments.

Hypothesis 3: Positive mixing effects on belowground nutrient cycling should increase under low water availability (Fig. B.5).

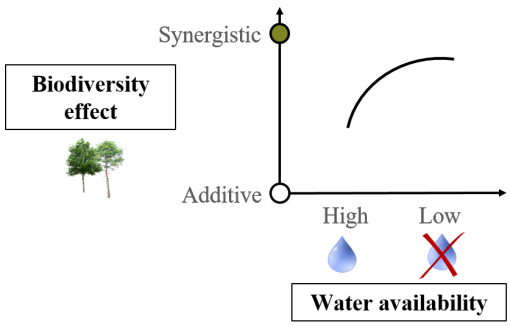

Figure B.5: Hypothesis for chapter 3.

Chapter 4: Nutrient use efficiency

Chapter 4 asks how water supply may influence the effect of tree diversity on nutrient use efficiency (NutUE) at the forest stand level. The definition hereby used for NutUE is the aboveground net primary productivity (ANPP) divided by the total amount of nutrients in the leaf litterfall (Vitousek 1982). Similar to the first three chapters, three sub-hypotheses were tested for chapter 4:

- ($H_{4.1}$) Mixing tree species should decrease NutUE in comparison to monocultures. This is because we expected an increase in soil nutrient availability in mixed species plots due to a greater leaf litter complementarity (Chapman et al. 2013; Handa et al. 2014; Talkner et al. 2009), which should in turn lead to lower retranslocation (Achat et al. 2018; Wright and Westoby 2003), and thus to an increase in litter nutrient concentrations. Because we expected similar increases in production in mixed species plots *via* isometric growth between aboveground net primary productivity and annual litterfall (Enquist and Niklas 2002), the increase in litter nutrient concentration with mixing would lead to a decrease in NutUE with mixing.

- ($H_{4.2}$) Irrigation should have a positive effect on stand NutUE due to higher growth (both ANPP and litter biomass) per unit of nutrient (Trichet et al. 2008; Vasconcelos et al. 2008).

- ($H_{4.3}$) The effect of mixing trees on NutUE should decrease with decreasing water availability, because we expected a strong positive diversity effect under low water supply on soil nutrient availability (Ratcliffe et al. 2017).

Hypothesis 4: Negative effect of mixing on NutUE should be more antagonistic under low water availability (Fig. B.6).

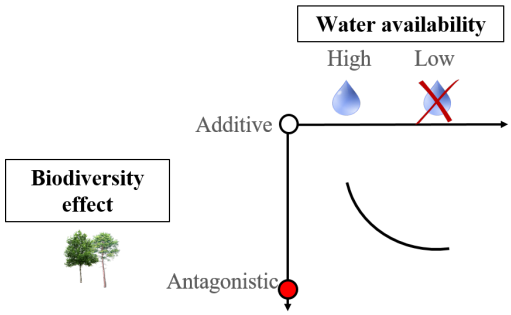

Figure B.6: Hypothesis for chapter 4.

B.2 Document organization

The first section of this thesis detailed the general context and a review of the literature on the effects of tree diversity and drought on nutrient cycling in forests (Section A). Then, I presented the main objective and the three main hypotheses of this thesis (Section B), after which I will briefly present the general methods (Section C). The following sections of this document are four chapters, in the format of a journal article, and a synthesizing discussion, to answer the main question of the thesis: **is there an interaction between tree diversity and water availability on nutrient cycling in forests?** For each section, a chapter introduction is included prior to the article, which reminds the reader of the general context, main objective and hypothesis, provides a general material and methods section, and summarizes the main results. References are also presented for each chapter.

Chapter 1 aimed to study the interactive effect of tree diversity and water availability along the soil profile, to test potential spatial complementarity on soil nutrient availability. To do so, soil cores were extracted from one for the field sites, which was divided into soil five layers down to 90 cm deep. At each layer, soil extracellular enzymes were assayed and available nutrients extracted and analyzed. The results of this study are presented in the following paper:

> Paper 1: **Maxwell, T.L.**, Augusto, L., Bon, L., Courbineau, A., Altinalmazis-Kondylis, A., Milin, S., Bakker, M.R., Jactel, H., & Fanin, N. (2020). Effect of a tree mixture and water availability on soil nutrients and extracellular enzyme activities along the soil profile in an experimental forest. *Soil Biology and Biochemistry*, 148, 107864.

In order to dive further into organic nitrogen breakdown dynamics, Chapter 2 focused on protein depolymerization, a key step to belowground nitrogen cycling. Soils were sampled in the upper 15 cm in July to test the potential interactive effect of mixing tree species and water availability on these rates. We then measured the microbial biomass and ran an isotopic pool dilution with the quantification of ^{15}N-labelled amino acids using a novel high throughput method.

> Paper 2: **Maxwell, T.L.**, Augusto, L., Tian, Y., Wanek, W., & Fanin, N. High water availability stimulates soil organic nitrogen breakdown, while tree mixing has little effect (In preparation for submission as a short communication).

Chapter 3 aimed to study the interactive effect of tree diversity and water availability, to test potential temporal complementarity on soil nutrient availability and uptake by trees. To do so, ion-exchange resins were installed for periods of six months, net mineralization

rates estimated using Raison's method throughout the vegetation season, and a ^{15}N soil labeling experiment took place at two time points, at budburst and during summer, whose fate was followed into the leaves to estimate uptake. The results are presented in the following paper, soon to be submitted:

> Paper 3: **Maxwell, T.L.**, Fanin, N., Munson, A.D., Lambrot, C., Scolan, L., Trichet, P., & Augusto, L. Tree species composition and nutrient uptake drive soil nutrient availability, even under contrasted levels of water availability (In preparation for submission as a primary research article).

Chapter 4 asked how water supply may influence the effect of tree diversity on nutrient use efficiency (NutUE) at the forest stand level. This study was conducted on two field experiments in temperate forests that manipulate *in situ* both tree diversity (*via* species richness), and water availability (by irrigation and partial rain exclusion). We collected leaf litter, measured tree aboveground net primary productivity (ANPP), and analyzed both green leaf and litter nutrient contents to calculate NutUE and nutrient resorption efficiency (NutRE) at each site. The results have been submitted and are under revision:

> Paper 4: **Maxwell, T.L.**, Fanin, N., Parker, W., Bakker, M.R., Belleau, A., Meredieu, C., & Augusto, L., Munson, A.D. Tree species dominance drives nutrient use efficiency in young mixed species plantations, at both high and low water availability (Under revision).

The general discussion of the thesis aims to bring together the results from the four separate chapters. This synthesis will elucidate the overall patterns of the tree diversity × water availability interaction on nutrient cycling as a whole. Finally, a conclusion is presented with the main results, implications, limitations, and perspectives.

C GENERAL METHODS

C.1 Presentation of the experimental sites

To answer our research question, we had access to two similar experimental platforms in France and in Canada, both of which are part of the worldwide Tree Diversity Network (TreeDivNet, Fig. C.1). These are two of the unique platforms which manipulate tree biodiversity and water resource. These "common garden" experiments enable us to specifically target species effects, in contrast to mixtures which developed under site conditions, which may have influenced tree nutrition and the identity of species developed in the specific locations. In both research sites, we focused on the effect of species richness as a measure for biodiversity. Both research sites are located in temperate forests with trees planted within their natural habitat range. The first three chapters are solely focused on the site in France, while the fourth chapter focuses on both sites. Setting up similar experimental designs on both platforms will additionally help us investigate the context-dependency of our biodiversity-drought interaction effects, to determine whether our results are generalizable across different forest ecosystems.

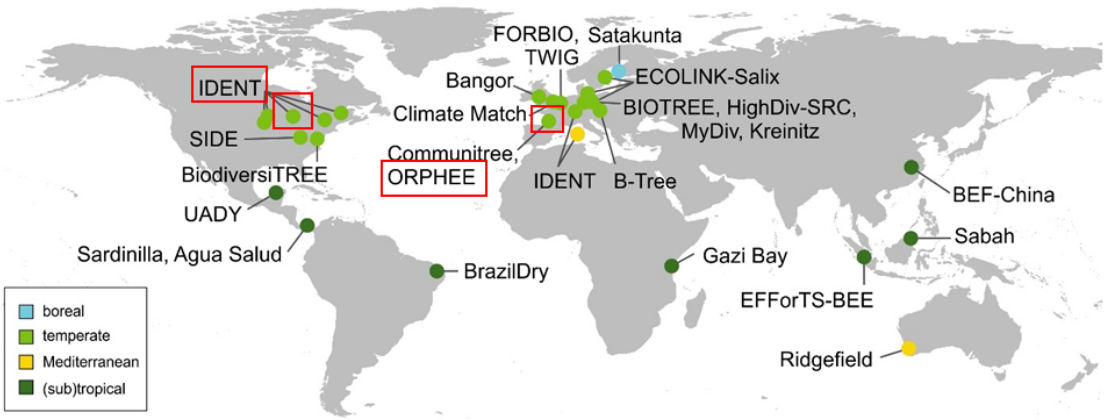

Figure C.1: Map of the TreeDivNet network. It is the largest network of biodiversity experiments worldwide, including around 1.1 million trees planted in the 25 experiments on a total surface area of 821 hectares. In the red boxes are the two sites that we will be studying: ORPHEE in southwestern France and IDENT-SSM in Sault-Ste-Marie, Ontario, Canada. More details can be found at the following website: http://www.treedivnet.ugent.be/.

C.1.1 ORPHEE, France

The first experimental site is the Observatoire Regional de PHEnologiE (ORPHEE) in southwestern France. It is located in the largest artificial forest in occidental Europe (*Landes de Gascogne*), which has an area of almost one million hectares. In this region, precipitation is expected to decrease by 10% and temperature is expected to increase by 1.5°C by 2100 (IPCC 2014; Loustau et al. 2005). As a consequence, the productivity of the maritime pine (*Pinus pinaster*) stands, which characterize this forest, is anticipated to decrease by 10% by 2050 due to these regional changes (Mora et al. 2014).

The tree plantation in ORPHEE occurred in January and February 2008 on a clear cut of former maritime pine stand, with a single plough and fertilizer application of 52.4 kg P ha^{-1} and 99.6 kg K ha^{-1} prior to plantation. The geographic location of the site is 44°44.35' N, 00°47.9' W and its elevation is 60m above sea level (Castagneyrol et al. 2013). The soils are over 95% sand, with a limited ability to retain water. A total of 256 plots were planted, each with 100 trees within a 20 m x 20 m area. The plots were designed with a diversity gradient of 1,2,3,4 and 5 species to study species richness effects, of which three species were deciduous (*Betula pendula*, *Quercus pyrenaica*, *Quercus robur*) and two were evergreen (*Quercus ilex*, *Pinus pinaster*). In total, there were 31 different combinations of plots, each in four control blocks and in four irrigated blocks (Fig. C.2). The location of the plots within the blocks was randomly determined and the plots were separated by three meters from one another.

The irrigation for half of the blocks started in 2014, and occurs seasonally from May to October to avoid frosts. The water is taken directly from the groundwater, which has low concentrations of nitrogen and phosphorus, but higher concentrations of other elements, such as calcium and magnesium (Jolivet et al. 2007). The equivalent of 3 mm of precipitation is sprayed per night by a 2m high jet installed in the center of each experimental plot. Each half block is irrigated sequentially. The 3 mm of water added daily corresponds to the average evapotranspiration of the region. The water table in the "*Landes de Gasgogne*" forest, where ORPHEE is located, is relatively shallow in wintertime, ranging from 40 to 80 cm below the surface. The mean average precipitation is 870 mm (1993-2018), but most precipitation occurs from the fall to the spring, leading to very dry summers in ORPHEE, which we consider to be representative of water stress. In order to characterize the water deficit in our plots, we will use the local precipitation and temperature data (CLIMATIK, Agroclim INRAE). Previous studies have also found differences in water conditions between the control and irrigated blocks at our site. In 2016, researchers found that the average gravimetric soil water content (measured in soil cores taken between 20 and 40 cm depth) in control blocks was about 7% lower than in the irrigated blocks (Rahman et al. 2018, Fig. 3B). More recently, colleagues

investigating predawn leaf water potential during a summer drought in 2018, found significant differences between the water availability treatments, indicating that irrigation partially relieved the water limitation at this time (Martin-Blangy 2021, Fig. III.2.2).

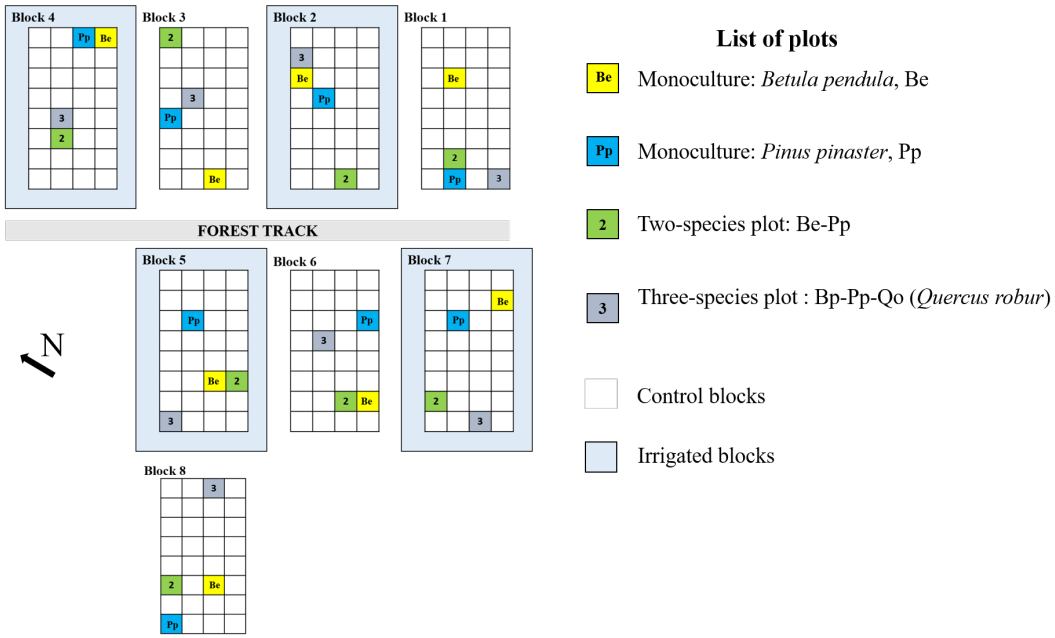

Figure C.2: Description of the ORPHEE experimental site in southwestern France. The monocultures of the maritime pine (*Pinus pinaster*) and silver birch (*Betula pendula*), and the mixture of the two composed of 50% of each, was studied. In Chapter 4, the 3-species mixture is additionally studied.

We chose to work closely with two species, silver birch (*Betula pendula*) and maritime pine (*Pinus pinaster*), two of the species that were successful in growing in the poor sandy and acidic soils of the *Landes of Gascogne*. Indeed, the three other planted species (all of the *Quercus* genus) were not yet tall enough to substantially interact with birch or pine. We measured our various variables in the monocultures of both these species, and in their mixed species plots. The 3-species plot (Bp-Pp-Qo, Fig. C.2) was additionally included in chapter 4. Our biodiversity experiment compares two-species mixtures to monocultures, which is a transition expected to yield the strongest yield of productivity (Pretzsch et al. 2013a). Because our site has a low soil fertility, we also expected a positive effect of species-mixing (Pretzsch et al. 2013a).

C.1.2 IDENT-SSM, Canada

The second site is in Sault-Ste-Marie, northern Ontario, Canada. It is one of the IDENT (International Diversity Experiment Network with Trees) sites, which are also part of the TreeDivNet network along with ORPHEE. In Canada, forests occupy 38% of the total land coverage with an area of 347 million hectares, which accounts for 9% of the total forests in the world. It contributes around $24.6 billion CAD to Canada's economy per year and 52% of the forest industry jobs are in Ontario and Quebec (*The State of Canada's Forests Report* 2018).

The tree plantation occurred in 2013 on a low-input abandoned agriculture field. The geographic location of the site is 46.546610° N, -84.455650° W, its elevation is 210 m at it is a sandy loam soil type (Belluau et al. 2021). A total of 9016 trees were planted among 184 plots which each measure 10.24 m² (Fig. C.3). Preliminary testing of soil fertility and pH were done to evaluate the fertilizer needs. The area was treated with herbicide, plowed, treated with 3 tons per hectare (t ha^{-1}) of calcitic limestone in April 2013 prior to planting and then in May 2013, 1.5 t ha^{-1} slow release NPK fertilizer was applied. The soil texture is between sandy loam and loamy fine, with an average of 8% clay, 12% silt and 80% sand. The mean average temperature is 4.7°C, with highs around 17°C in the summer, and the mean average precipitation is 898 mm (1981-2010), which is approximately evenly distributed throughout the year.

The plots are divided amongst 8 blocks, four of which are weekly irrigated from June 1 to August 31. The irrigation started in June 2014 and consists of a weekly spraying of the equivalent of 2.54 cm of rain (double the normal quantity) over the course of four hours. The other four blocks have a rain exclusion system, which are gutters that are installed every year from May 1 to October 15, imposing a 25% rainfall exclusion (Belluau et al. 2021, Fig. S2 & S3). Gutters with holes in them were also installed in the irrigated blocks to remove the effects of the presence of the gutter (i.e. shade). There are 23 different species combination plots which create orthogonal gradients in species richness and functional diversity (3 levels). There are six total species (*Pinus strobus* (Ps), *Betula papyrifera* (Ba), *Acer saccharum* (As), *Picea glauca* (Pg), *Larix laricina* (Ll), *Quercus rubra* (Qu)) with a diversity gradient of 1, 2, 4 and 6 species. We chose to work with five monocultures (Ps, Ba, As, Ll, Qu), along with four two-species plots (Ba-Ps, Ba-As, Ba-Ll, Qu-Ll), and two four-species plots (Qu-As-Ll-Ps, Ba-As-Ll-Ps) in order to study the effect of species richness. The plots are randomly distributed within each block and maintained in a weed-free condition by hand.

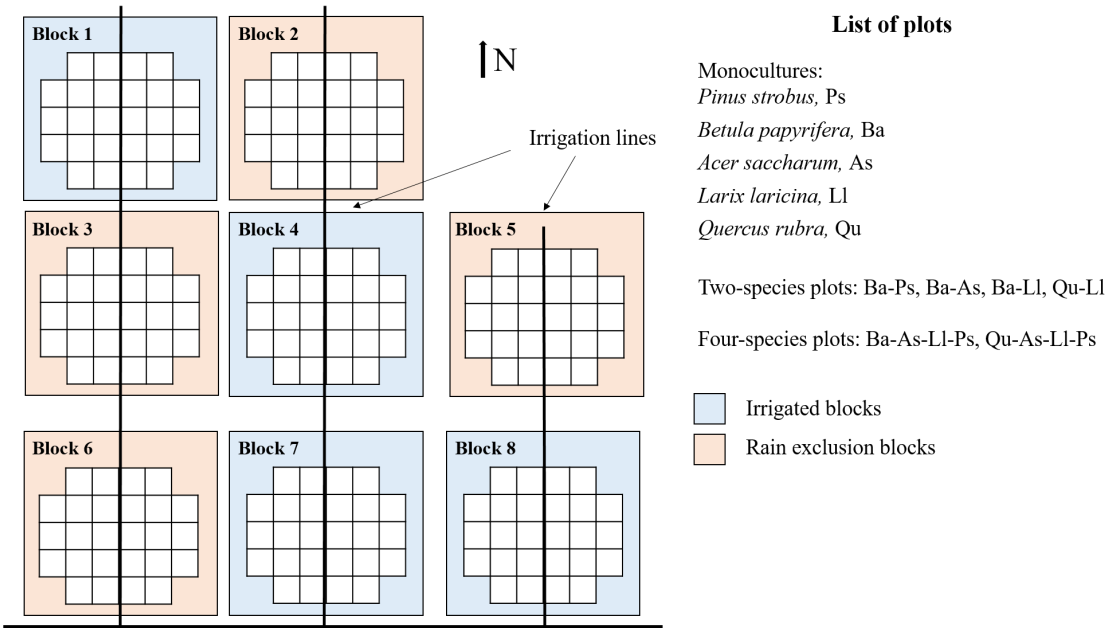

Figure C.3: Description of the IDENT-SSM experimental site in Sault-Ste-Marie, Canada. We studied five monoculture plots, four 2-species, and two 4-species plots out of the 26 possible plots. Plots are randomly distributed within each block. Exact placement can be found in Annex E.3.

C.1.3 Site characteristics

Both sites are located in temperate forests with species within their climatic and geographic distribution (Table C.1). They both have the tree species combinations of pure birch, pure pine, and mixed birch-pine. Both pine species (*Pinus pinaster* and *Pinus strobus*) are calcifuge, with a preference for well-drained or sandy soils, and which establish at the early succession stage. Both birch species (*Betula pendula* and *Betula papyrifera*) are comparable deciduous species, as they are pioneer species and relatively short-lived, preferring mesic to slightly xeric sites and siliceous soils.

Table C.1: Comparison table of both experimental sites in 2019.

	IDENT-SSM, Canada	ORPHEE, France
Tree plantation year	2013	2008
Mean annual precipitation (mm)	899	870
Mean annual temperature (ºC)	4.7	12.5
Soil texture	sandy loam ∼loamy fine sand	sandy
Soil pH [H_2O]	5.4	4.5
Plot size	49 trees in 10.24 m²	100 trees in 400 m²
Blocks (water availability treatment)	4 rain exclusion (low H2O), 4 irrigation (high H_2O)	4 control (low H_2O), 4 irrigation (high H_2O)
Number of species	1, 2, 4	1, 2, 3
Species composition	*Betula papyrifera* (Ba), *Pinus strobus* (Ps), *Acer saccharum* (As), *Larix laricina* (Ll), *Quercus rubra* (Qu), Ba-Ps, Ba-As, Ba-Ll, Qu-Ll, Qu-As-Ll-Ps, Ba-As-Ll-Ps	*Betula pendula* (Be), *Pinus pinaster* (Pp), *Quercus robur L.* (Qo), Be-Pp, Be-Pp-Qo

C.2 Methods

The methods used in each chapter will be briefly presented in this introductory section of the thesis. More detail about the methods can be found in each respective chapter.

C.2.1 Chapter 1: Spatial complementarity of soil nutrient availability

The main objective in Chapter 1 was to investigate the interactive effect between tree diversity and water availability on soil nutrient availability along the soil profile. More specifically, soil cores were sampled at the ORPHEE field site, and available nutrients and soil extracellular enzyme activity (EEAs) were measured at five soil depths (0-5, 5-15, 15-30, 30-60, and 60-90 cm). To do so, fresh soil was extracted with H_2O and available nutrients (NH_4^+, NO_3^-, PO_4^{-2}) were analyzed by colorimetry. On frozen aliquots of same soil samples, the potential activity of seven hydrolytic soil enzymes was measured: enzymes that catalyze the degradation of organic carbon (β-1,4-glucosidase [BGLU]), 1,4-β-D-cellobiohydrolase [CBH], α-1,4-glucosidase [AGLU], and β-xylosidase [XYL]), nitrogen (β-1,4-N-acetyl-glucosaminidase [NAG], L-leucine aminopeptidase [LAP]), and phosphorus (acid phosphatase [AP]) (Bell et al. 2013; Fanin et al. 2016b).

Figure C.4: Photos depicting the study system and sample collection for chapter 1. (a) Example of pure birch stands, (b) example of pure pine stands, (c) sample collection with a mechanical driller to approximately 1 m depth, (d) example of soil cores in which the OM color varied from dark brown for the upper layer to yellow in the deep layers. Photos: N. Fanin. Figure from Fanin et al. 2021.

C.2.2 Chapter 2: Soil organic nitrogen breakdown

The main purpose of this chapter was to investigate the interactive effects of mixing tree species and water availability on soil organic nitrogen processes using an isotope pool dilution method (Wanek et al. 2010, Fig. C.5). We collaborated with the developers of the method at the Terrestrial Ecosystem Research lab at the University of Vienna, Austria, to measure these rates on soil samples (0-15 cm) taken in July 2020 at the ORPHEE experimental site. The total free amino acid (TFAA) concentrations in the extracts were measured (Jones et al. 2002), to calculate the tracer addition volume and concentration (^{15}N-AA 98‰, Cambridge Isotopes). The isotope pool dilutions were done on two aliquots of fresh soil, one stopped after 15 min and the other 45 min after tracer addition using cold 1 M KCl. Ammonium was removed from the extracts by microdiffusion. Free amino acids in the ammonium-free extracts were then converted to N_2O gas which was analyzed by purge-and-trap isotope ratio mass spectrometry (PT-IRMS) for the isotopic composition and concentration of the amino-acids (Lachouani et al. 2010; Noll et al. 2019a). Using the change of ^{15}N-^{14}N amino acids over time, organic N breakdown rates were calculated (Kirkham and Bartholomew 1954).

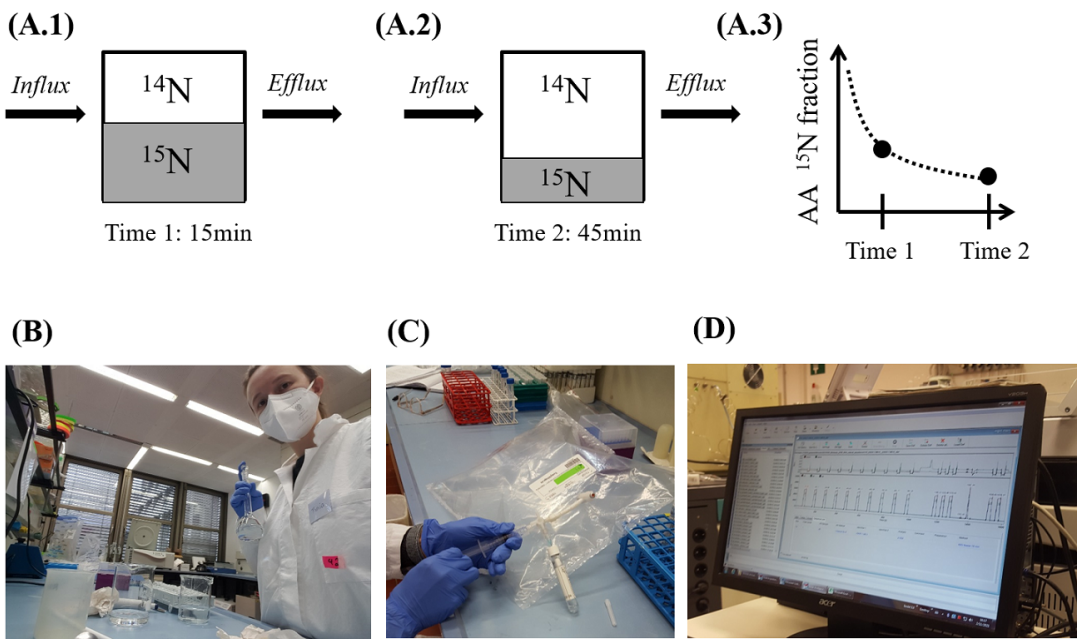

Figure C.5: Principles of the novel high throughput isotope pool dilution method. (A.1, A.2) The fraction of ^{15}N in amino acids (AA) found for the 1st assay, stopped 15 min after adding the tracer, and for the 2nd assay, stopped after 45 min. (A.3) The evolution of the ^{15}N fraction: the tracer is quickly consumed, and then decreases at a constant rate between the two time points. (B) Removal of NH_4^+ using microdiffusion, conversion of AA to N_2O gas using oxidation-reduction reactions. (C) Transfer of N_2O gas to clean and helium-filled exetainers. (D) Measurement of peaks to find ^{15}N:^{14}N ratio in the N_2O on a PT-IRMS.

C.2.3 Chapter 3: Temporal complementarity of soil nutrient availability and uptake

The main objective of Chapter 3 was to investigate the interactive effect between tree diversity and water availability to test potential temporal complementarity on soil nutrient availability an uptake by trees (Fig. C.6). To do so, we placed ion-exchange resins (Binkley and Matson 1983), which captured the long-term nutrient accumulation over four periods of 6 months, for a total of two years. In order to better understand the mechanisms behind nutrient accumulation in the resins during the vegetation period, we installed *in situ* soil incubations following Raison et al. 1987. We were able to estimate mineralization, root uptake and leaching rates during these shorter incubation periods from April to September of the second year. Finally, we applied a ^{15}N labelled solution to the soil in order to estimate nutrient uptake at budburst and at peak phenology in early summer.

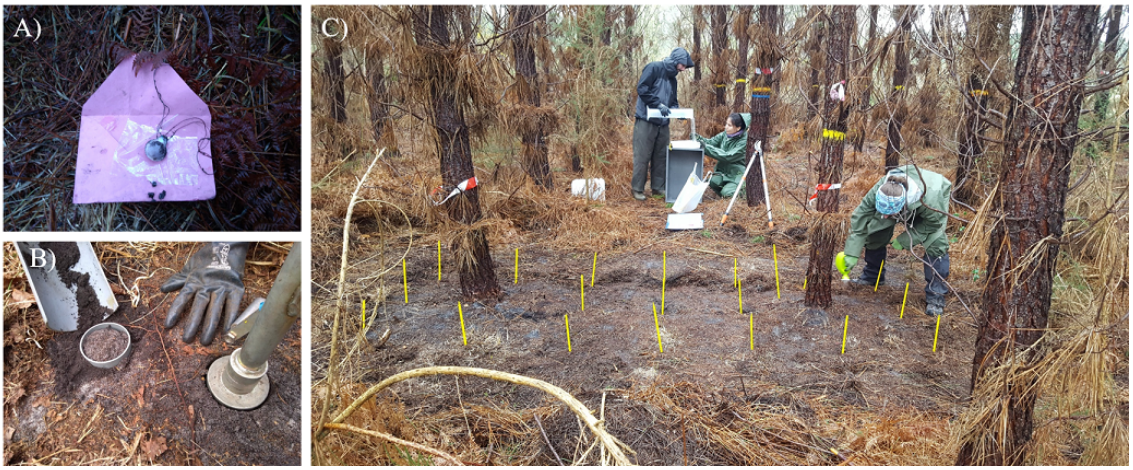

Figure C.6: Images of the three methods and the experimental site. A) An ion-exchange resin after removal, B) incubation tube and soil corer for non-incubated soil analyses, and C) soil labeling using a small watering can in measured quadrants within a 3 m^2 zone.

C.2.4 Chapter 4: Nutrient use efficiency

The main objective in Chapter 4 was to investigate how water supply may influence the effect of tree diversity on nutrient use efficiency (NutUE) at the forest stand level (Fig. C.7). This study was conducted on two field experiments in temperate forests that manipulate *in situ* both tree diversity (*via* species richness), and water availability (by irrigation and partial rain exclusion). We considered the NutUE equation as ANPP divided by the total amount of nutrients in the leaf litterfall (Vitousek 1982). Therefore, leaf litter was regularly collected over a year using litter traps, then dried, sorted and weighed. An aliquot of the leaf litter and green leaf samples (collected at peak vegetation in the summer) were ground up to measure nutrient concentrations. The aboveground net primary productivity (ANPP) was calculated as the difference in aboveground biomass between the two years of litterfall monitoring. Finally, nutrient resorption efficiency was also measured as the percent of nutrients which are remobilized from the leaves (prior to leaf abscission) into developing tissues or stored for later use (Wright and Westoby 2003).

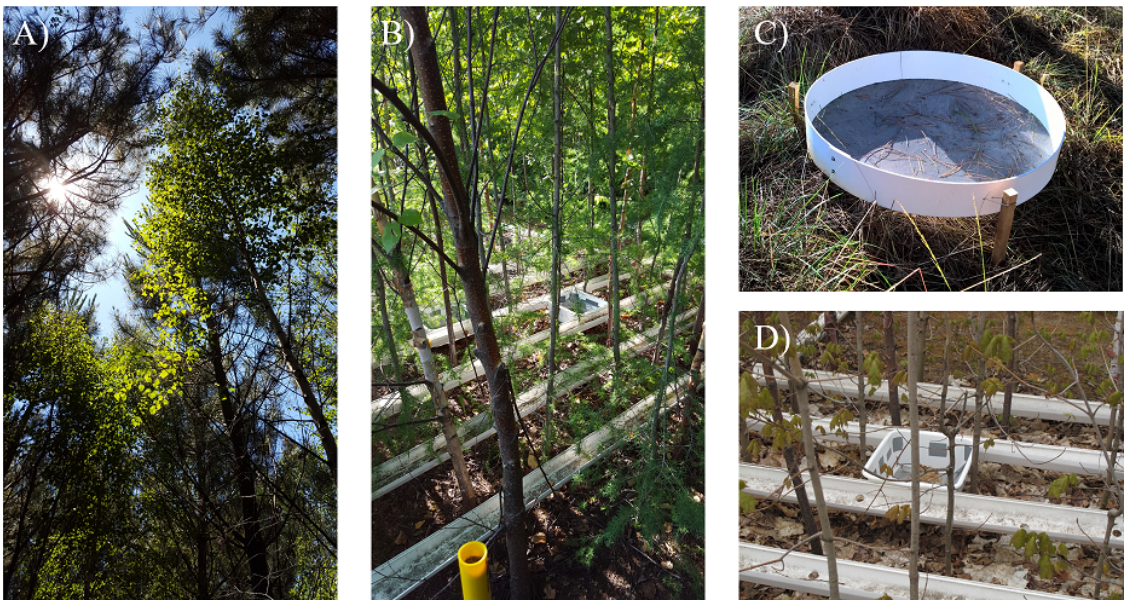

Figure C.7: Experimental sites for chapter 4. The birch-pine mixtures and litter traps at the A) & C) ORPHEE and B) & D) IDENT-SSM field sites.

CHAPTER ONE

Effect of a tree mixture and water availability on soil nutrients and extracellular enzyme activities along the soil profile in an experimental forest

Published as: Maxwell, T.L., Augusto, L., Bon, L., Courbineau, A., Altinalmazis-Kondylis, A., Milin, S., Bakker, M.R., Jactel, H., & Fanin, N., 2020. Effect of a tree mixture and water availability on soil nutrients and extracellular enzyme activities along the soil profile in an experimental forest. *Soil Biology & Biochemistry* 148, 107864.

Abstract

An increasing number of studies demonstrate that tree species biodiversity can affect primary productivity and nutrient cycling in forests due to several factors, such as complementarity, facilitation or selection effects. For instance, resource partitioning in soils has been found to allow a more optimized nutrient uptake in mixed species plots compared with monocultures. However, how these effects will be modified by climate change – such as water availability – is not as well understood, especially in deep soil layers. Therefore, we specifically asked how water availability may influence the effect of tree mixtures on soil microorganism activity by measuring extracellular enzyme activities (EEAs) and available nutrients along the soil profile (down to 90 cm) in a 10-year-old plantation in southwestern France, which manipulates tree species composition (*Pinus pinaster* and *Betula pendula*, in monocultures and in mixed plots) and irrigation. Our results showed that EEAs directly depend on tree species composition and water conditions in interaction with soil depth; we found a positive effect of mixing birch and pine on carbon (C)- and nitrogen (N)-related EEAs at an intermediate soil depth (15–30 cm soil layer), while the effect of increasing water availability increased phosphorus (P)-related EEAs mostly in the upper soil layers (0 to 30 cm). However, we found no significant interactive effect between tree diversity and water availability on EEAs, underlining that the negative effect of lower water availability cannot be offset by the positive effect of mixing tree species. Differences in EEAs and available nutrients along the soil profile highlight the importance to look at different soil depths to better understand how nutrient cycling may be affected by increasing tree diversity and changes in water availability.

Keywords: biodiversity, drought, nutrients, precipitation, soil extracellular enzyme activity

Résumé

Ce chapitre vise à étudier comment la disponibilité en eau peut influencer l'effet du mélange d'arbres sur l'activité des microorganismes du sol en mesurant les activités des enzymes extracellulaires (AEE) et les nutriments disponibles le long du profil du sol (jusqu'à 90 cm) dans une plantation d'arbres de 10 ans dans le sud-ouest de la France. Nous avons trouvé un effet positif du mélange de bouleau et de pin sur les AEE liés au carbone (C) et à l'azote (N) à une profondeur de sol intermédiaire (15-30 cm), tandis que l'effet de l'irrigation a augmenté les AEE liés au phosphore (P) principalement dans les couches supérieures du sol (0 à 30 cm). Cependant, nous n'avons trouvé aucun effet interactif significatif entre diversité des arbres et irrigation, ce qui sous-entend que l'effet négatif d'une plus faible disponibilité en eau ne peut être compensé par un effet positif du mélange des espèces d'arbres.

Mots clés : activité enzymatique extracellulaire du sol, biodiversité, nutriments, précipitations, sécheresse

Chapter Introduction

Spatial complementarity

General context

An important step of carbon (C), nitrogen (N), and phosphorus (P) cycling is the decomposition of organic matter that derives from leaf and root litter (Hättenschwiler et al. 2005; Zechmeister-Boltenstern et al. 2015). Among other processes involved in decomposition of organic matter, soil microorganisms contribute to its degradation by releasing extracellular enzymes to acquire energy and resources by degrading adjacent soil organic matter (Burns 1982; Burns et al. 2013). Studying soil extracellular enzyme activities (EEAs) can thus provide information on potential belowground microbial mineralization processes and how microbial communities drive C, N, and P cycling in soils (Allison et al. 2007; Fanin et al. 2016b; Henry 2013, Fig. 1.1).

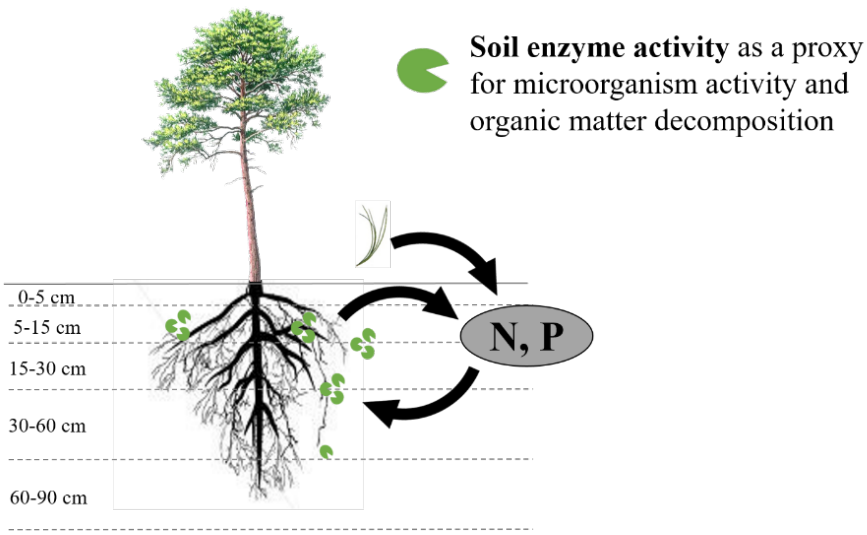

Figure 1.1: Soil enzyme activities as a part of belowground nutrient cycling.

Tree species may have important effects on soil EEAs due to their varying litter qualities (Weand et al. 2010). Recently, Alberti et al. 2017 found a positive synergistic effect of tree diversity on EEAs, due to the influence of varying litter traits on soil C and N mineralization. These results suggest that an increase in tree species richness may influence biogeochemical cycling due to stimulation of microbial activity (Hacker et al. 2015; Steinauer et al. 2014). Soil EEAs are also measured to assess microbial responses

to global changes, including precipitation changes in varying ecosystems (Henry 2013). EEAs have been found to be negatively affected by water stress (Brockett et al. 2012; Schlesinger et al. 2016), including in forests (Sardans and Peñuelas 2005). However, these effects may vary with soil depth due to water runoff dynamics and infiltration from topsoil to deeper soils.

Few studies have looked into tree biodiversity effects on belowground productivity and soil functioning (Alberti et al. 2017; Brassard et al. 2013; Khlifa et al. 2017; Ma and Chen 2016; Strukelj et al. 2021), and we know little about how limiting water conditions may affect biodiversity benefits, and if their interactive effects may affect soil microbial activity and biogeochemical cycling in mixed forests.

Objective and hypotheses

The primary objective of this chapter was to investigate the interactive effect between tree diversity and water availability on available nutrients and soil extracellular enzyme activities (EEAs) along the vertical soil profile. To do so, we sampled soils at five depths in the ORPHEE experimental plantation (where both tree diversity and water availability were manipulated) and tested three hypotheses.

First, we tested the hypothesis ($H_{1.1}$) that EEAs would be higher in a two-species mixture than in the corresponding pure plots since mixing different litter qualities may lead to more synergistic litter decomposition (Chapman et al. 2013; Cornwell et al. 2008). Specifically, we hypothesized that the effect of mixing trees on EEAs should be higher in the topsoil layer, where the effect of tree species identity is highest (Augusto et al. 2002) and where the organic matter quantity is highest and richest in nutrients (Jobbágy and Jackson 2001; Melillo et al. 1989).

Secondly, we hypothesized ($H_{1.2}$) an increase in water supply would have a positive effect on EEAs by alleviating the water constraint (Sardans and Peñuelas 2005; Xiao et al. 2018). We anticipate that the effect of increasing water availability should be highest in the topsoil, mainly because microbial communities at the soil surface are often more subjected to water stress than those living in deeper soil horizons (Fanin et al. 2020).

Finally, we hypothesized ($H_{1.3}$) that the positive effect of mixing two tree species on EEAs would be strongest in the plots experiencing the dry summers. This is because mixing tree species with contrasting root exploration strategies may favor drought resistance through facilitation mechanisms (Bello et al. 2019).

Experimental design

A total of 4 soil cores were taken from 3 species composition treatments (pure birch, pure pine, mixed birch-pine) in 2 water availability treatments, each replicated 4 times in the ORPHEE experiment (Fig. 1.2). Each soil core was divided into the 5 soil layers (0–5, 5–15, 15–30, 30–60, and 60–90 cm), making thus a total of 480 samples collected for nutrient and enzyme analyses.

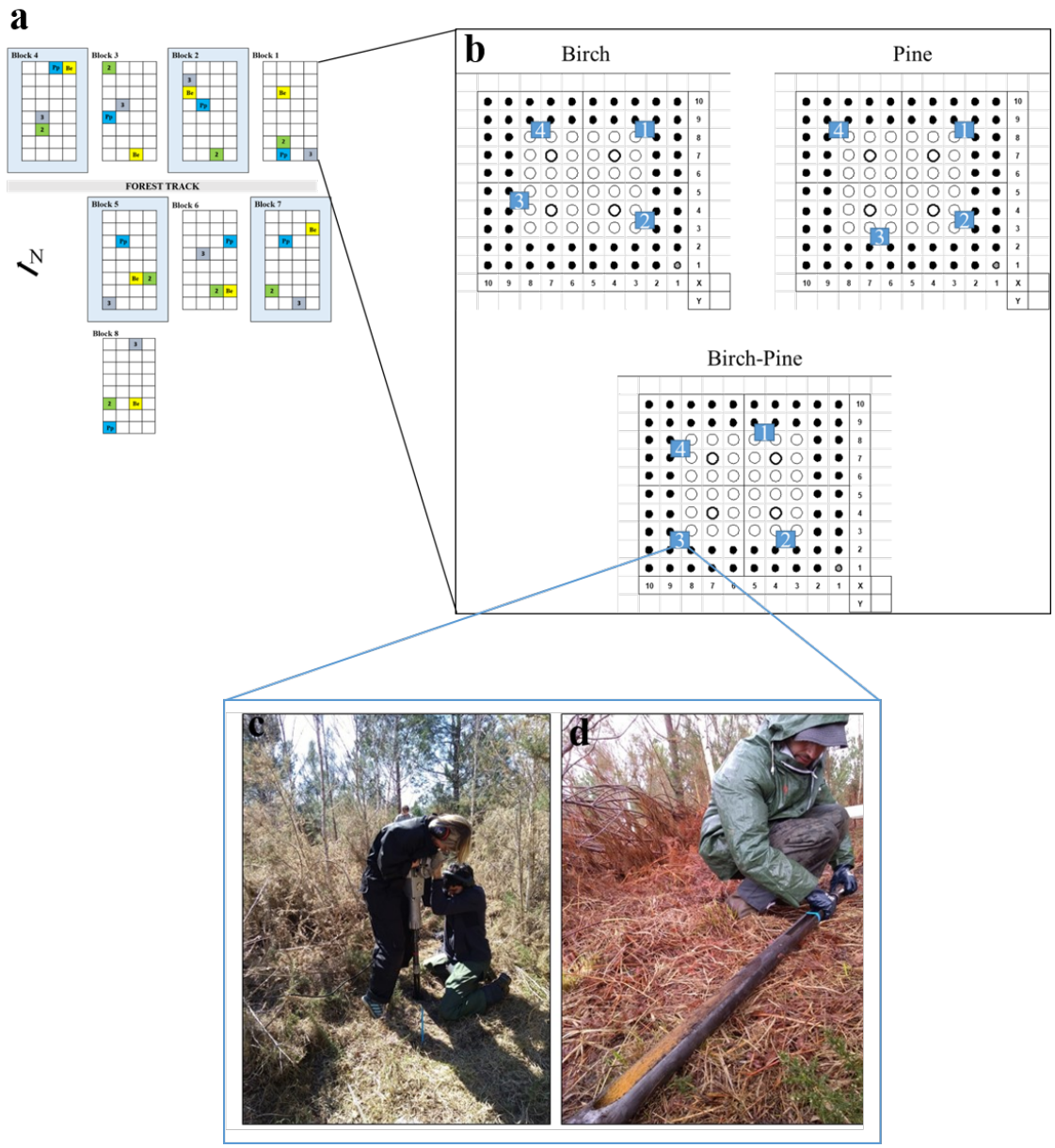

Figure 1.2: Schematic representation of the experimental design. (a) Eight blocks at the ORPHEE experimental site, four control and four irrigated, (b) Three species composition at the plot level (400 m²) in each block, with the location of the four soil cores per plot (example given for Block 1), (c) Using a mechanical driller to sample the deeper soil layers, and (d) sample deep soil core.

Main results

Our results from the experiment showed that soil extracellular enzyme activities (EEAs) directly depended on tree species composition and water conditions in interaction with soil depth. First, we found a positive effect of mixing birch and pine on carbon (C)- and nitrogen (N)-related EEAs (Hypothesis $H_{1.1}$) only at an intermediate soil depth (15–30 cm soil layer). The non-significant effect of mixing in the topsoil may have been due the strong presence of tree and understory roots in the 0-15cm soil layer. Secondly, the effect of increasing water availability increased phosphorus (P)-related EEAs mostly in the upper soil layers (0-30 cm; $H_{1.2}$).

However, in contrast to our expectations ($H_{1.3}$), we found no significant interactive effect between tree diversity and water availability on EEAs, indicating that the negative effect of lower water availability cannot be offset by the positive effect of mixing tree species.

Additionally, we found a strong positive correlation between the available P and the C_{enz}:P_{enz} ratio. This implies that when P availability is high, microorganisms are strongly limited by C, inciting them to produce more C-related enzymes to get access to the energy produced by decomposing organic matter.

Take-home message

This study confirmed that there was vertical spatial complementarity of belowground microorganism activity at the mid-soil layer in the temperate podzol of the ORPHEE experimental site, but that this effect was not impacted by changes in water availability.

1 Paper 1: Effect of a tree mixture and water availability on soil nutrients and extracellular enzyme activities along the soil profile in an experimental forest

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Pure pine and pure birch trees at ORPHEE on April 1st 2019. Photo: T. L. Maxwell.

1.1 Introduction

Forest biodiversity provides a multitude of ecosystem goods and services (Gamfeldt et al. 2013; Jactel et al. 2017; Liang et al. 2016). However, forests in many areas are threatened by more intense and frequent drought periods (IPCC 2014; Schwalm et al. 2012), with important negative consequences such as on nutrient cycling (He and Dijkstra 2014; Schlesinger et al. 2016) or even on tree survival (Hartmann et al. 2018). A well-explored option to mitigate these negative effects is to increase tree stand diversity (Lebourgeois et al. 2013; Pretzsch et al. 2013b). Indeed, mixed-species forests generally have been found to be more productive than pure stands (Baeten et al. 2019; Cardinale et al. 2007; Liang et al. 2016; Zhang et al. 2012) and are more stable to climatic fluctuations due to overyielding, species asynchrony and favorable species interactions (Jucker et al. 2014a; Kardol et al. 2018; Morin et al. 2014). However, recent studies have shown that the positive effect of tree diversity on forest productivity might be annihilated under drought conditions (Jactel et al. 2018). In addition, not all tree species mixtures are more resistant to water stress (Grossiord 2020). Although many studies on biodiversity effects focused on aboveground plant productivity, only a few addressed belowground productivity and soil functioning (Alberti et al. 2017; Brassard et al. 2013; Ma and Chen 2016). Furthermore, we know little about how limiting water conditions may affect biodiversity benefits and if their interactive effects may affect soil microbial activity and biogeochemical cycling in mixed forests.

An important step of carbon (C), nitrogen (N), and phosphorus (P) cycling is the decomposition of organic matter that derives from leaf and root litter (Hättenschwiler et al. 2005; Zechmeister-Boltenstern et al. 2015). Among the main mechanisms involved in decomposition, one process is based on extracellular enzymes (Sinsabaugh et al. 2002). Soil microorganisms release extracellular enzymes to acquire energy and resources by degrading neighboring soil organic matter (Burns 1982; Burns et al. 2013). Studying soil extracellular enzyme activities (EEAs) can thus give information on potential belowground microbial mineralization processes and how microbial communities drive C, N, and P cycling in soils (Allison et al. 2007; Fanin et al. 2016b; Henry 2013). Soil EEAs are measured to assess microbial responses to global changes, including precipitation changes in varying ecosystems (Henry 2013). EEAs have been found to be negatively affected by water stress (Brockett et al. 2012; Schlesinger et al. 2016), including in forests (Sardans and Peñuelas 2005). A recent global meta-analysis concluded that drought led to a significant decrease in urease and phenol oxidase activity, while an increase in precipitation significantly increased N-related enzyme activities (Xiao et al. 2018). However, these effects may vary with soil depth due to water runoff dynamics and infiltration from topsoil to deeper soils. Topsoil is thus expected to be more positively affected by an increase in precipitation due to its increase in soil moisture, a major factor determining EEAs in soils

(Brockett et al. 2012), whereas deeper soils may be less affected by precipitation, have less varying moisture and thus less effect on EEAs (Fanin et al. 2020).

Tree species may also have important effects on EEAs due to their varying litter qualities (Weand et al. 2010). Recently, Alberti et al. 2017 found a positive synergistic effect of tree diversity on EEAs, due to the influence of varying litter traits on soil C and N mineralization. These results suggest that an increase in tree species richness may influence biogeochemical cycling due to stimulation of microbial activity (Hacker et al. 2015; Steinauer et al. 2014). Because tree species diversity may positively influence microbial biomass and activity (Chen et al. 2019a; Lucas-Borja et al. 2012; Thakur et al. 2015; West et al. 2006), and that both microbial biomass and substrate pools decrease with depth (Agnelli et al. 2004; Fierer et al. 2003), there is a need to study the effect of tree diversity on EEAs along the soil profile (Herold et al. 2014; Loeppmann et al. 2016; Stone et al. 2014). The diversity effects may also directly depend on root biomass along the litter-soil continuum. Mixing tree species with contrasting root systems may lead to niche partitioning along the soil profile (Brassard et al. 2013), which can benefit exploration of the total soil volume (Schmid and Kazda 2002) and total nutrient uptake (Richards et al. 2010). In addition, facilitation mechanisms between associated species, such as hydraulic lift (Zapater et al. 2011), may be especially advantageous in mixed stands during drought events. Thus, investigating how tree diversity may offset the negative effects of drought along the soil profile would provide new insights into how biodiversity effects on nutrient cycling may change in a future climate.

In this study, our primary objective was to investigate the interactive effect between tree diversity and water availability on available nutrients and EEAs along the soil profile. In practice, we sampled soils at five depths in an experimental plantation where both tree diversity and water availability (through irrigation) were manipulated and tested three hypotheses. First, we tested the hypothesis (H_1) that EEAs would be higher in a two-species mixture than in the corresponding pure plots since mixing different litter qualities may lead to more synergistic litter decomposition (Chapman et al. 2013; Cornwell et al. 2008). Specifically, we hypothesized that the effect of mixing trees on EEAs should be higher in the topsoil layer, where the effect of tree species identity is highest (Augusto et al. 2002) and where the organic matter quantity is highest and richest in nutrients (Jobbágy and Jackson 2001; Melillo et al. 1989). Secondly, we hypothesized (H_2) an increase in water supply would have a positive effect on EEAs by alleviating the water constraint (Sardans and Peñuelas 2005; Xiao et al. 2018). We anticipate that the effect of increasing water availability should be highest in the topsoil, mainly because microbial communities at the soil surface are often more subjected to water stress than those living in deeper soil horizons (Fanin et al. 2020). Finally, we hypothesized (H_3) that the positive effect of mixing two tree species on EEAs would be strongest in the plots experiencing the dry

summers. This is because mixing tree species with contrasting root exploration strategies may favor drought resistance through facilitation mechanisms (Bello et al. 2019).

1.2 Materials and methods

1.2.1 Experimental site and sample collection

We conducted this study at the ORPHEE experiment in southwestern France, 40 km southwest of Bordeaux (44°44.35' N, 00°47.9' W, altitude of 60 m asl) (Castagneyrol et al. 2013). It is part of the international Tree Diversity Network (TreeDivNet) and is located in the largest artificial forest in occidental Europe (*Landes de Gascogne*). The mean annual temperature is 12.5°C and the mean average precipitation is 870 mm (1993-2018), but most precipitation occurs from the fall to the spring, leading to very dry summers, which we consider representative of water stress. The former vegetation was a mature *Pinus pinaster* stand located on a flat area. The tree plantation in ORPHEE occurred in 2008, after a clear-cut of the pine forest, with a single plough and fertilizer application of 60 kg of P_2O_5 and 60 kg of K_2O per hectare prior to plantation. The predominant soil type is a podzol characterized by a coarse texture (95% sand) and low soil fertility (Augusto et al. 2010), particularly in phosphorus (Trichet et al. 2008). The water table is relatively shallow in wintertime, generally ranging from 40 to 80 cm below the soil surface. A total of 256 plots were planted representing five species (*Betula pendula* Roth., *Pinus pinaster* Soland. in Aït., *Quercus ilex* L., *Quercus pyrenaica* Willd., *Quercus robur* L.) in all possible combinations from one (monocultures) to five species mixtures. Each plot has 100 trees within a 20 m by 20 m area. Trees are planted in a substitutive (same tree density irrespective of the composition) alternate (trees from one species have neighbors from all other associated species) pattern. The plots are separated by 3 m from one another, and randomly located within eight blocks: four control (dry) blocks and four irrigated blocks. The irrigation was started in 2015, and occurs seasonally from May to October to alleviate summer stress. The start and end dates of irrigation differ slightly between years depending on meteorological and technical constraints. The water is taken directly from the groundwater, which has low concentrations of nitrogen and phosphorus, but higher concentrations of other elements, such as calcium and magnesium (Jolivet et al. 2007). A 2 m tall sprinkler installed in the center of each experimental plot sprays every day the equivalent of 3 mm of precipitation. On average for the three summers preceding our sampling, control blocks received 318 mm of water from early May to late September, while irrigated blocks received 777 mm of water during the same period. When using both temperature and precipitation (+ irrigation) to determine arid periods in an ombrothermic diagram (Walter and Lieth 1967), control blocks are subjected to more stress than irrigated blocks (Fig. S1.1).

Among all of the plots, we chose to work with three species composition treatments: pure silver birch (*Betula pendula* Roth.) plots, pure maritime pine (*Pinus pinaster* Soland. in Aït.) plots, and the mixed birch-pine plots, which we used as a proxy to study tree diversity effects. These two species were selected because, contrary to the three other tree species, they grew fast despite the poorness of the sandy soil, which enabled the building of fairly dense forest stands. In 2018, the average tree height was about 8 m for the two species. The understory was mostly composed of Bracken, Molinia, Gorse, and ericaceous shrubs, with a few minority species (Besom heath, Buckthorn, Bramble, Honeysuckle). As a whole, the understory layer represented less than 1% of the total aboveground biomass and the diversity was similar across all treatments, with on average six to seven vascular species per plot.

We sampled plots in all eight blocks (4 irrigated, 4 dry) from March 19th to 21st 2018. In each plot, we collected samples at four different locations, each surrounded by four trees, i.e. four pines, four birches, or two pines and two birches. The forest floor (layer of litter above the soil mineral layers) was collected using a quadrant (10 cm × 20 cm). Samples were dried at 40°C until weight stabilization and ground. At each sampling location, we also collected a soil core down to 0.9 m depth in two steps. The top 0–15 cm of soil was collected manually with a soil corer (8 cm width). The bottom 15–90 cm of soil was collected with a mechanical drill, attached onto an auger (4 cm width). Afterwards, the soil cores were divided into five layers: 0–5, 5–15, 15–30, 30–60, and 60–90 cm. Soil samples were immediately placed in coolers and transported to the nearby lab, where each sample was sieved to 2 mm and homogenized. The roots were cleaned and stored in 10% alcohol until further analyses. One soil subsample was kept at 4°C for immediate extraction of available nutrients, and a second subsample was stored at -20°C for enzyme assays. Soil moisture was assessed for each sample by comparing the fresh to the dry soil weights after at least 72 h at 50°C. There were thus a total of 480 samples collected for nutrients, enzymes, soil moisture and root analyses (3 species composition × 2 water levels × 4 blocks × 5 depths × 4 replicates, Fig. S1.2). Composites of the four cores were made for each soil layer per plot, and these samples were then air-dried for chemical analysis (3 species compositions × 2 water levels × 4 blocks × 5 depths = 120 samples).

In addition to the soil samples, we collected litterfall sampled from litter traps that were installed in all plots in three blocks per water treatment at the beginning of the 2017 fall season. Two circular traps were installed in each plot: the traps were plastic cylinders with a height of around 20 cm, a metal net on the inferior opening, and a surface area of 0.716 m^2. We collected the litter in November 2017, made one composite per plot, dried the samples at 40°C until weight stabilization and then ground them up (3 species compositions × 2 water levels × 3 blocks = 18 samples). The C:N ratios were determined after measuring total C and N by dry combustion (NF ISO 10694 and 13878).

1.2.2 Available nutrients

After sample collection, available ammonium (NH_4^+), nitrate (NO_3^-), and phosphate (PO_4^{-2}) were measured in one aliquot of all the soil samples, corresponding to 480 analyses (Fig. S1.2). In brief, 15 mL of ultrapure water was added to a glass vial with a 3 g aliquot of the soil sample, which was then shaken for 16 h at 4°C. Water extraction was favored over *KCl* extraction due to potential interference of the extractant with available phosphate in sandy podzols (Achat 2009), and because it has a similar extraction efficiency for mineral N as in saline solutions (Gallet-Budynek, personal communication). The solution was placed vertically at ambient temperature for an hour to enable sedimentation, after which the supernatant was transferred into a tube through a Whatman filter of 8µm mesh size. The solution was left at ambient temperature for 3 h, and then filtered a second time with a syringe equipped with a 5 µm mesh paper. The final solution extractant was poured into scintillation tubes to measure NO_3^-, NH_4^+ and PO_4^- by colorimetry (San++, Automated Wet Chemistry Analyzer, Breda, Netherlands). The total available mineral N was expressed as the sum of mineral N from NO_3^- and NH_4^+ (µg N g^{-1} dry soil) and available mineral P as PO_4^{-2} (µg P g^{-1} dry soil).

1.2.3 Enzyme assays

In the same samples previously described for available nutrients, on the other aliquot stored frozen at -20°C, we measured the potential activity of seven hydrolytic soil enzymes. We measured enzymes that catalyze the degradation of organic carbon (β-1,4-glucosidase [BGLU]), 1,4-β-D-cellobiohydrolase [CBH], α-1,4-glucosidase [AGLU], and β-xylosidase [XYL]), nitrogen (β-1,4-N-acetyl-glucosaminidase [NAG], L-leucine aminopeptidase [LAP]), and phosphorus (acid phosphatase [AP]) (Bell et al. 2013; Fanin et al. 2016b). Briefly, 2.75 g of the thawed soil sample was homogenized in 91 ml of 50 mM sodium acetate buffer in a blender for 1 min. Soil slurries of two technical replicates were then added to a 96-deepwell (800 µl) microplate using an eight-channel electronic pipette (Eppendorf Xplorer Plus, Hamburg, Germany). Additional quench control replicates of the soil slurry, 4-methylumbelliferone or 7-amino-4-methylcoumarin standard curves (200 µl of respectively 0-100 µM and 0-10 µM concentrations) and controls without substrate addition (soil + 200 µl water), were included with each sample. Soil slurries were incubated with fluorometric substrates for 3 h at 25°C. After the incubation period, plates were centrifuged for 3 min at 3000 rpm, after which 250 µl of supernatant was transferred from each well into a black flat-bottomed 96-well plate. The fluorescence was measured by a microplate reader (Synergy H1 microplate reader, Biotek, Winooski, USA) using an excitation wavelength at 365 nm and emission wavelength at 450 nm. From the fluorescent values and standard calibration curves, each enzyme activity was calculated

and converted to nmol g^{-1} dry soil h^{-1}, and then converted to mmol kg^{-1} to express the enzymes as quantities. We then summed the BGLU, CBH, AGLU, and XYL quantities for total C-related enzymes (hereafter referred to as C_{enz}), and the NAG and LAP quantities for the total N-related enzymes (in the following referred to as N_{enz}). We refer to the acid phosphatase (AP) quantity as P_{enz}. The ratios of total enzymes were subsequently calculated, which we refer to as C_{enz}:N_{enz} , C_{enz}:P_{enz}, and N_{enz}: P_{enz}. This is a tool that has been frequently used to measure ecological stoichiometry and microbial limitations (Fanin et al. 2016b; Sinsabaugh et al. 2008; Stone et al. 2014). However, differences between potential enzyme activities and enzyme responses to nutrient additions have recently been found to be ambiguous, so some care must be taken when interpreting enzyme ratios as growth-limiting factors (Rosinger et al. 2019).

1.2.4 Root biomass

Roots were extracted from the same soil samples in which available nutrients and enzymes were analyzed. Root extraction consisted of sieving the collected samples and then watering the remaining roots to clean them and remove the soil. The live roots were sorted and separated into the different species. A representative portion of each root sample was oven-dried for 4 days at 40°C and weighed. For each soil sample, the root biomass data of both the tree species (pine and birch) and the understory species were used in our statistical analyses to determine their effect on the potential enzyme activities in a redundancy analysis (see section 2.6).

1.2.5 Soil chemical analyses

The subsequent soil analyses were done at the plot level by making composites of the four soil cores at each of the five depths along the soil profile (3 species compositions × 2 water levels × 4 blocks × 5 depths = 120 samples). The soil pH was determined in a 1:10 soil:water suspension. The C:N ratios were determined after measuring total C and N by dry combustion (NF [i.e., French standard] ISO [i.e., international standard] 10694 and 13878; AFNOR 1999). The particle size distribution was analyzed for each block (one composite sample per block and per soil layer), using the fractionation method without decarbonatation (for five fractions, i.e., clay, fine silt, coarse silt, fine sand, and coarse sand) according to French standards (AFNOR 1999).

Table 1.1: Selected properties of the study site in the three different tree species compositions, each in both dry control and irrigated blocks. Data are mean ± standard error of the mean (n = the total number of samples per treatment).

		Birch		**Birch + Pine**		**Pine**	
	n	Control	Irrigation	Control	Irrigation	Control	Irrigation
Litterfall		39.88 ±	50.96 ±	99.50 ±	110.19 ±	149.39 ±	160.43 ±
C:N	3	3.69	3.47	21.78	27.54	9.22	4.22
Forest floor		35.85 ±	37.42 ±	42.15 ±	61.00 ±	51.58 ±	69.61 ±
C:N	4	0.88	2.78	4.61	4.79	2.95	8.05
Soil moisture		13.21 ±	12.79 ±	12.46 ±	12.86 ±	12.54 ±	13.91 ±
(%)	16	0.45	0.45	0.61	0.76	0.81	0.63
Soil pH		4.50 ±	4.62 ±	4.45 ±	4.46 ±	4.49 ±	4.67 ±
[H_2O]	4	0.04	0.06	0.05	0.04	0.05	0.17

Note: Litterfall was collected in the fall, while forest floor, soil moisture and soil pH were collected in early spring. Three blocks of each water availability treatment were sampled for litterfall while four blocks were sampled for the other variables. We analyzed one composite sample per plot for litterfall, forest floor and pH or four replicates per plot for soil moisture. Weighted means were calculated for soil pH and soil moisture along the soil profile and detailed data per soil depth layer can be found in Table S1.2

1.2.6 Statistical analyses

All of the statistical analyses were done using R software (version 3.5.1). First, extreme values were removed following the outlier labelling rule with a conservative tuning parameter of g = 2.2 (Hoaglin and Iglewicz 1987). On average, 1.25% of data were removed. Weighted means, using soil weight per layer, were used when presenting values per soil column.

Mixed models were used to assess the effect of the following fixed effect factors: tree species composition (pure birch, pure pine, mixed birch-pine), water availability (irrigation, control), soil depth (five depths as levelled factors) and their interaction, on the enzyme activities and available nutrients. Block was included as a random factor to account for the spatial structure of our experimental design. Plots were then nested within the block random factor to enable a comparison of treatments within each block separately. Finally, the sampling replicate number was nested within the plot to account for the non-independency of soil horizons within a soil core of a certain plot. Soil moisture was added as a co-variable in the mixed model. We then applied contrasts and performed post-hoc tests Tukey HSD post-hoc tests, with $\alpha = 0.05$ used as the level to define significance.

We calculated non-additive effects of mixing birch and pine trees by comparing observed values in the mixed-species plots to expected values, calculated as the mean enzyme quantities of the component species in the monocultures (Barantal et al. 2014). We used a Welch two sample t-test to test whether the observed enzyme quantities differed from the expected enzyme quantities for control and irrigated treatments. The percentage increase in enzyme quantities (the relative mixture effect) was calculated by ((observed - expected)/expected)

* 100, according to (Wardle et al. 1997). Then, we used a redundancy analysis (RDA) to elucidate the relationships between individual enzymes and their respective biochemical variables (i.e. available nutrients, root biomass, and soil characteristics), included as the so-called environmental variables. We also included enzyme ratios as supplementary variables in the RDA. Finally, we used Mantel tests with Spearman's rank correlations (n = 999 permutations) to quantify the relationship between enzyme activities and environmental variables.

Table 1.2: Results of the linear mixed effects models to test for the effects of tree species composition (pure birch, pure pine, mixed birch + pine), water availability (irrigation, dry control), soil depth and their interactions on C-related EEAs (sum of XYL, AGLU, BGLU, and CBH), N-related EEAs (sum of NAG and LAP), P-related EEA (AP), available N and available P. Soil moisture was used as a covariable in the model and not shown here. Significant results are in bold font.

Variables	**C-related EEAs**				**N-related EEAs**				**P-related EEA**			
	num DF	*den DF*	*F*	*p*	*num DF*	*den DF*	*F*	*p*	*num DF*	*den DF*	*F*	*p*
Species (S)	2	12	2.87	0.096	2	12	0.95	0.414	2	12	0.26	0.774
Irrigation (I)	1	6	4.89	0.069	1	6	**6.91**	**0.039**	1	6	1.38	0.285
Depth (D)	4	350	**159.74**	**<.0001**	4	349	**103.86**	**<.0001**	4	351	**155.38**	**<.0001**
S × I	2	12	0.82	0.464	2	12	2.20	0.153	2	12	0.10	0.908
S × D	8	350	**7.20**	**<.0001**	8	349	**2.45**	**0.014**	8	351	**2.31**	**0.020**
I × D	4	350	1.88	0.113	4	349	1.77	0.134	4	351	**2.51**	**0.042**
S × I × D	8	350	0.58	0.794	8	349	0.43	0.901	8	351	0.20	0.990
Variables					**Available N**				**Available P**			
					num DF	*den DF*	*F*	*p*	*num DF*	*den DF*	*F*	*p*
Species (S)					2	12	3.30	0.072	2	12	0.48	0.631
Irrigation (I)					1	6	0.05	0.835	1	6	0.36	0.570
Depth (D)					4	351	**20.65**	**<.0001**	4	350	**49.21**	**<.0001**
S × I					2	12	3.08	0.083	2	12	0.14	0.869
S × D					8	351	1.66	0.107	8	350	1.60	0.123
I × D					4	351	**6.16**	**<.0001**	4	350	0.45	0.775
S × I × D					8	351	1.33	0.228	8	350	1.35	0.216

1.3 Results

1.3.1 Soil chemical properties

Soil texture did not vary between irrigated and control blocks with on average 95% sand, 2% silt, and 3% clay (Table S1.1). Soil pH increased down the soil profile in all plots (Table S1.2), and varied according the interaction between soil depth and plot species composition ($p < 0.05$). Additionally, soil moisture varied between depths in all plots ($p < 0.001$, Table S1.2), with a decreasing trend from the top to the bottom soil. Averaged across all species treatments, soil moisture was significantly higher in the irrigated blocks than in the control blocks for the 0–5 cm ($p < 0.05$) and the 30–60 cm layers ($p < 0.01$) (Table S1.2), despite that the soil sampling was done in March, a period when the irrigation was switched off.

The forest floor C:N ratio was significantly higher in irrigated than in control blocks ($p < 0.05$, Table 1.1). The forest floor C:N in the pure birch was significantly lower than that in the mixed species plots ($p < 0.01$) and the pure pine plots ($p < 0.001$, Table 1.1). Irrigation increased the litterfall C:N ratio in the three different species compositions (Table 1.1).

1.3.2 Enzyme activities and available nutrients

There was a significant effect of the interaction between the plot species compositions and soil depth for the C-related EEAs ($p < 0.0001$), N-related EEAs ($p < 0.05$), and P-related EEA ($p < 0.05$) (Table 1.2). In both pure birch and pure pine plots, C- and N-related EEAs decreased steadily from the 0–5 cm to the 60–90 cm layer (Fig. 1.3A). However, the pattern was different in mixed birch and pine plots: there was a sharp increase in EEAs from the 5–15 cm layer to the 15–30 cm layer. At this 15–30 cm mid soil layer, C-related EEAs were significantly higher than those in pure plots, with an increase on average of 77% (Fig. 1.3A), and N-related EEAs were significantly higher (+49%) than in pure pine plots ($p < 0.05$) (Fig. 1.3B). This resulted in synergistic, non-additive effects at this intermediate soil layer (Fig. 1.4): the effect of mixing birch and pine was however restricted to this soil depth, and was independent of irrigation with a similar increase in both control and irrigated blocks for C- and N-related EEAs. In contrast, the P-related enzyme activity decreased with depth similarly in all species compositions, but was 24% lower in the mixed plot at the 30–60 cm layer compared with the monocultures (Fig. 1.3C). There were more C- and N-related EEAs in pure birch than pure pine plots at the 0–5 cm layer in control blocks, but no overall significant differences were observed along the soil profile between the monocultures for each of the C-, N- and P-related EEAs.

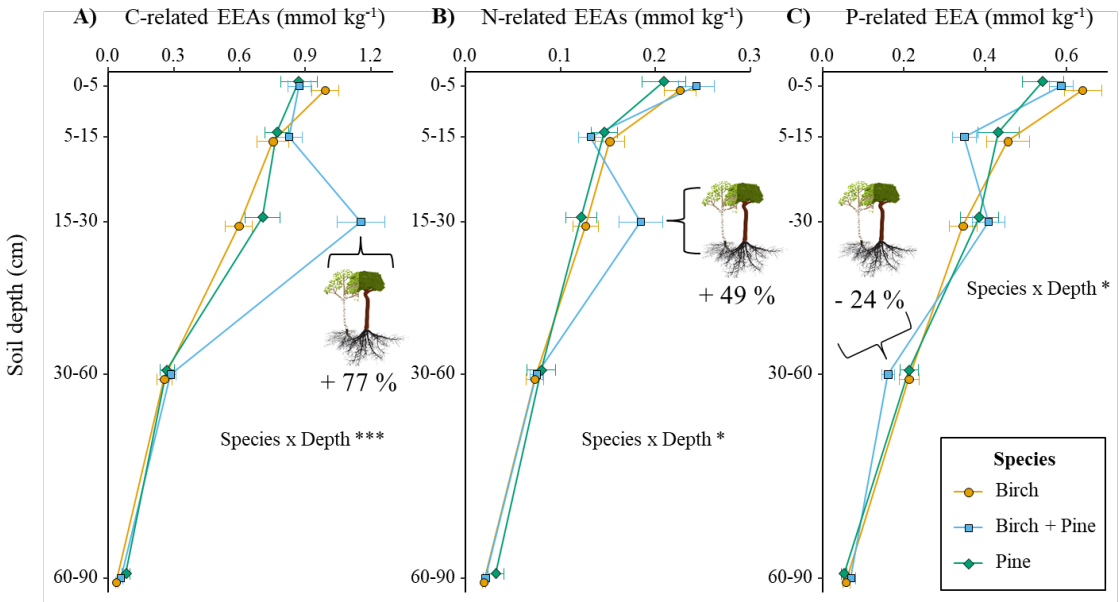

Figure 1.3: Distribution of the extracellular enzyme activities (EEAs) along the soil profile in pure birch (circles, *yellow*), mixed birch + pine (squares, *blue*) and pure pine plots (diamonds, *green*); A) C-related EEAs (sum of XYL, AGLU, BGLU and CBH); B) N-related EEAs (sum of NAG and LAP); and C) the P-related EEA (AP). The water availability treatments were pooled for each species composition treatment. Data points represent means at each depth interval of the four replicates per plot, in each of the eight blocks (n = 32) and error bars represent standard errors of the mean.

Our results also showed an overall positive effect of irrigation on N-related EEAs (Table 1.2): averaged among species treatments along the soil profile, irrigation increased N-related EEAs by 28% compared with control blocks (Fig. 1.5A). From the 0–5 cm to the 5–15 cm soil layer, there was a sharp decrease of N-related EEAs in both control and irrigated blocks. These values remained steady down to the 15–30 cm layer, after which there was another steady decline down to the 60–90 cm layer. For P-related EEAs, the overall enzyme pattern was similar to N-related EEAs, with a steady decline down to the 60–90 cm layer. However, the significant effect of irrigation on the P-related EEA depended on soil depth (Table 1.2): AP activity in irrigated blocks tended to be higher only in the first 30 cm, with an increase on average of 20% compared with control blocks Fig. 1.5B).

Although total N varied in the top 0–5 cm soil layer from an average of 1.06 mg N g^{-1} in control blocks to 1.26 mg N g^{-1} in irrigated blocks (data not shown), we found that available N was relatively low in both water treatments (Fig. 1.6). N availability was interactively affected by soil depth and irrigation ($p < 0.0001$): adding water decreased available N by 40% in the first soil horizons layers (0–15 cm) while it increased available N by 55% in depth (30–90 cm) (Fig. 1.6A). Available P also differed along soil depth (Table 1.2). It increased from 0–5 to 5–15 cm in all plots and then steadily decreased until the 60–90 cm layer (Fig. 1.6B).

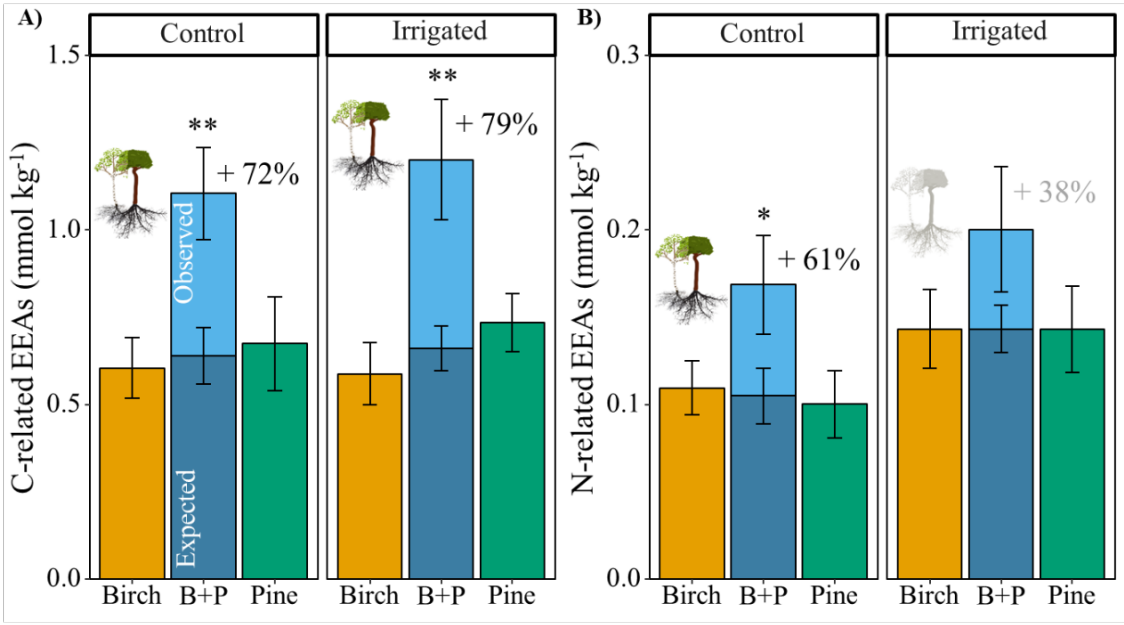

Figure 1.4: Synergistic non-additive effect of mixing birch and pine in the 15–30 cm soil layer on extracellular enzyme activities (EEAs). Bars represent observed means for the four replicates in each species compositions in the four blocks per water treatment (n = 16) and error bars represent standard errors of the mean for pure birch (*yellow*), observed mixed birch + pine (B + P, *light blue*) and pure pine plots (*green*). The expected additive effect of mixing two tree species (*dark blue*), calculated as the average value between observed pure plots, is superposed onto the observed values of mixing the two tree species. Significant values come from Welch two sample t-tests between the expected and observed values for the mixed species plot. A) C-related EEAs (sum of XYL, AGLU, BGLU, and CBH); B) N-related EEAs (sum of NAG and LAP).

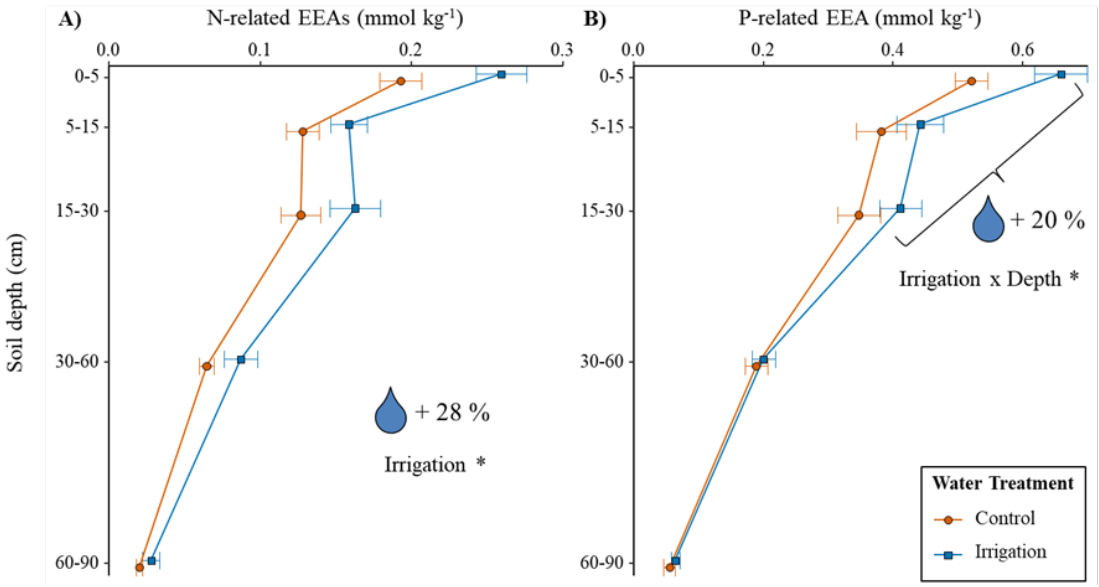

Figure 1.5: Distribution of the extracellular enzyme activities (EEAs) along the soil profile in dry control blocks (circles, *red*) and irrigated blocks (squares, *blue*) blocks; A) N-related EEAs (sum of NAG and LAP); and B) the P-related EEA (AP). The tree species composition treatments were pooled for each water availability treatment. Data points represent means at each depth interval of the four replicates in each of the three plots (species compositions) in the four blocks per water treatment (n = 48) and error bars represent standard errors of the mean.

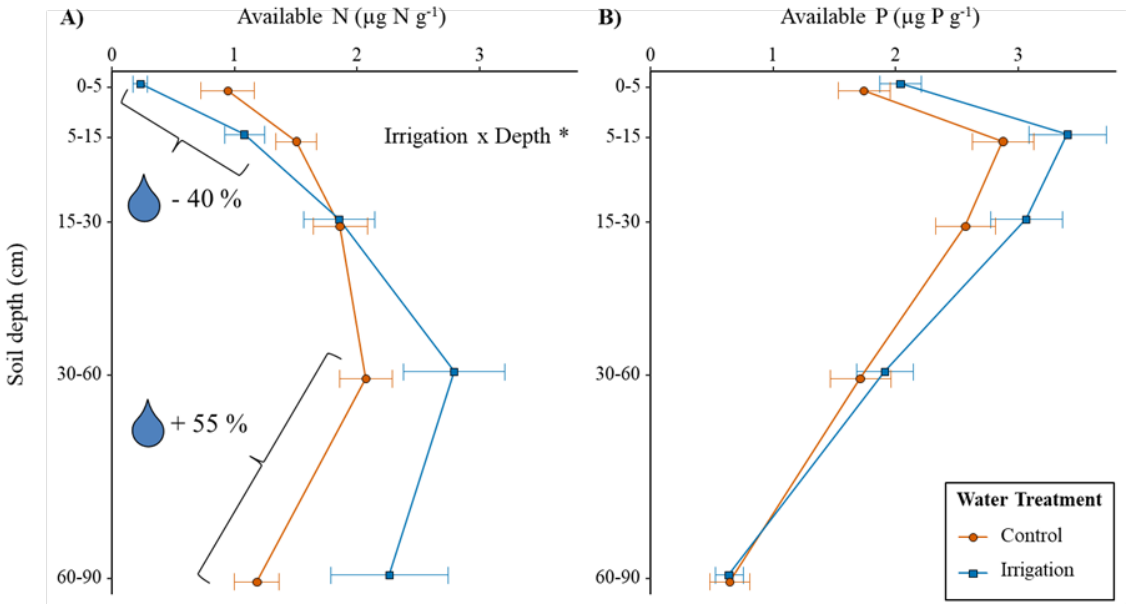

Figure 1.6: Distribution of the available nutrients along the soil profile in dry control blocks (circles, *red*) and irrigated blocks (squares, *blue*); A) available N (µg N g^{-1}); and B) available P (µg P g^{-1}). The tree species composition treatments were pooled for each water availability treatment. Data points represent means at each depth interval of the four replicates in each of the three plots (species compositions) in the four blocks per water treatment (n = 48) and error bars represent standard errors of the mean.

1.3.3 Environmental effects

Mantel tests showed that the seven individual hydrolytic enzymes were significantly correlated with the available nutrients and the belowground root traits (ρ = 0.569, p < 0.001). Biplots from the RDA distinguished the C-related EEAs (XYL, BGLU, AGL, CBH) from the N-related EEAs (NAG, LAP) and P-related EEA (AP) along the second axis, which weakly represented the variance of the system (Fig. 1.7). Available N was negatively related to the N-related EEAs along the first axis. The total tree species' fine root biomass within a plot and soil moisture, were also negatively related to the available N. The C-related EEAs fitted closely with the available P, which was unrelated to the AP enzyme. Both the C_{enz}:P_{enz} and C_{enz}:N_{enz} ratios (supplementary variables in green, Fig. 1.7) were closely related to the available P. The understory species fine root biomass within a plot species composition was generally unrelated to the individual enzyme activities.

As a whole, we found no correlation between the available N and the C_{enz}:N_{enz} ratio (adj. R^2 = 0.003, p = 0.12, Fig. 1.8A). However, we found a highly significant positive correlation between the available P and the C_{enz}:P_{enz} enzyme ratios (adj. R^2 = 0.24, p < 0.0001, Fig. 1.8B). This relationship was consistent across the three species compositions and water availability treatments.

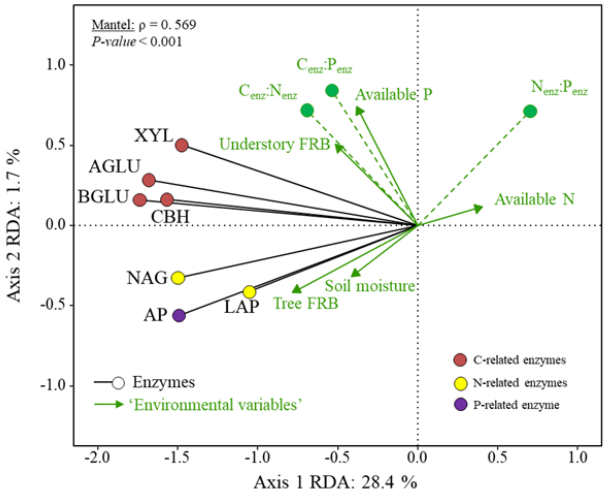

Figure 1.7: Redundancy analysis (RDA) ordination biplot of the seven hydrolytic extracellular enzyme activities (circles: C-related = *red*, N-related = *yellow*, P-related = *purple*), and of the environmental parameters (available P, available N, tree fine root biomass, understory fine root biomass, and soil moisture; arrows, *green*). The continuous supplementary variables were not included in the biplot calculation but are represented on the RDA (C_{enz}:N_{enz}, C_{enz}:P_{enz} and N_{enz}:P_{enz} ratios; circles, *green*), (n = 457).

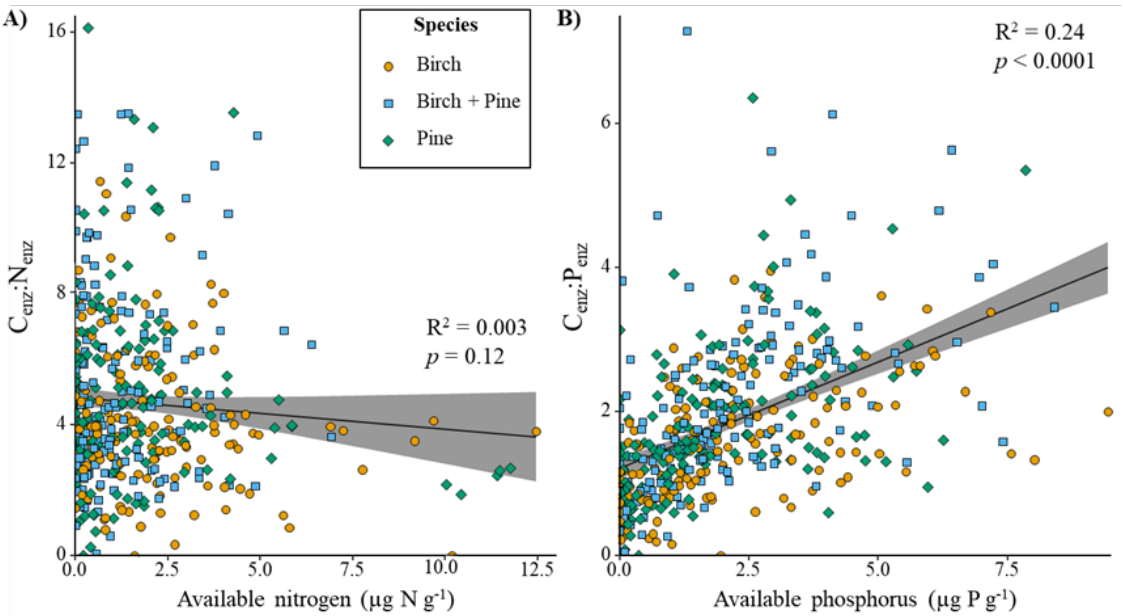

Figure 1.8: Relationship between available nutrients and enzyme ratios in the pure birch (circles, *yellow*), mixed birch and pine (squares, *blue*) and pure pine plots (diamonds, *green*), (n = 464). A) Non-correlation between available nitrogen and the ratio between the C-related enzymes and the N-related enzymes (C_{enz}:N_{enz}); B) Positive correlation between the available phosphorus and the ratio between the C-related enzymes and the P-related enzyme (C_{enz}:P_{enz}). The water availability treatments were pooled for each species composition treatment.

1.4 Discussion

By using a 10-year-old plantation which manipulates tree species composition and irrigation in southwestern France, we investigated the effect of mixing birch and pine, water availability, and their interaction on seven hydrolytic extracellular enzyme activities (EEAs) and available nutrients along the soil profile. Our results are partly in agreement with our two first hypotheses, thereby demonstrating that EEAs directly depend on tree species composition and water conditions. In contrast to our last hypothesis, we did not find any effect of the interaction between water availability and species mixing on EEAs, underlying that the negative effect of lower water availability cannot be offset by the positive effect of mixing tree species.

1.4.1 Effect on mixing tree species

In agreement with our first hypothesis, we found that mixing birch and pine had a positive effect on C- and N-related EEAs in interaction with soil depth (Table 1.2). However, contrary to our expectations that this effect should be more pronounced in the topsoil (Alberti et al. 2017), we observed a strong synergistic increase in C-related and N-related EEAs in the 15–30 cm soil layer compared to both birch and pine monocultures (Fig. 1.3, Fig. 1.4). While the litter quality was significantly higher in birch than in pine plots (lower C:N ratio, Table 1.1), the EEAs in the pure birch plots were only slightly higher than those in the pure pine plots (Fig. 1.3). Although we expected an influence of tree species identity (Purahong et al. 2016a) and litter quality on microbial decomposition and EEAs (Sinsabaugh et al. 2002), the little influence of the tree species on EEAs has been found in other studies. For instance, Herold et al. 2014 found no differences between coniferous and deciduous central European forests on six of our measured EEAs. Likewise, Weand et al. 2010 described no tree species effect on C-, N- and P-related enzymes in five single-species plots in an American Northeastern hardwood forest. This was also true for the N- and P-related EEAs studied in five tree species in a temperate secondary forest in Northeastern China (Diao et al. 2020). Therefore, it is unlikely that the increase in EEAs at the mid-soil layer of the mixed birch-pine plot was related to the different and complementary foliar litter quality of the two species. Instead, we interpret our results as a consequence of the higher nutrient demand of mixed stands.

Indeed, higher tree productivity in mixed stands may stimulate soil microbial biomass through an increase in root exudate quantity and diversity (Steinauer et al. 2016), or favor C allocation to ectomycorrhizal fungi to get N from organic molecules to sustain tree biomass production (Fernandez and Kennedy 2016; Lindahl and Tunlid 2015). Alternatively, niche partitioning in root systems or increase in root biomass along the soil profile may have

increased nutrient uptake (Jose et al. 2006), which may exacerbate, in turn, nutrient limitation of microorganism communities (Kuzyakov and Xu 2013). In both cases, this implies that there is likely a strong demand by microorganisms to degrade the soil organic matter (Fatemi et al. 2016) at this middle soil layer in mixed stands, and indicates that the potential effects of tree diversity on EEAs are not only limited to the superficial soil layers.

Contrary to C- and N-related EEAs, we found that there was a decrease in the P-related EEA in the 30–60 cm soil layer in the mixed-species plots compared to both birch and pine monocultures (Fig. 1.3C). This may be due to a shift in the nutrient needs of the microorganisms (Mooshammer et al. 2014) or because the changes in tree species diversity may lead to a shift in the structure of microbial communities (Liu et al. 2018; Pei et al. 2016; Schweitzer et al. 2008). For instance, changes in fungi to bacteria ratios may alter the stoichiometric requirements at the community scale (Fanin et al. 2013), which could explain the reduced and different effect of tree diversity on N- and P-related EEAs. On the other hand, this may be caused by differential root uptake and competition for the available nutrients between microorganisms and both tree and understory roots (Kuzyakov and Xu 2013; Richardson and Simpson 2011). This hypothesis is supported by the tendency of available N and P to decrease and increase respectively in mixed plots (Fig. S1.3), although the overall effect of tree species for the entire soil column was non-significant (Table 1.2). Taken together, these findings suggest that there is a shift in microbial C:N:P stoichiometry from monocultures to the two-species mixture along the soil profile.

1.4.2 Effect of water availability

In accordance with our second hypothesis, we found an increase in both N- and P-related EEAs with irrigation. Overall, EEAs were lower in the control blocks (Table S1.3), which were subject to the long dry summers of southwestern France (Fig. S1.1). Although we sampled in early spring, i.e. several months after the irrigation had been switched off, we still found differences in soil moisture between control and irrigated blocks (Table 1.1). The legacy of sub-optimal water conditions for the past eight years in the control blocks may have led to a decrease in both microbial biomass (Brockett et al. 2012) and tree and understory root biomass (Table S1.2; Brunner et al. 2015). The lower EEAs in these dry control blocks could thus be due to fewer microorganisms and roots, leading to less root exudation and enzyme stimulation (Zhang et al. 2019). This interpretation is supported by our RDA results, where tree fine root biomass points in the same direction as soil moisture (Fig. 1.7), demonstrating a positive covariation between these variables. The drought episodes experienced by control blocks and not by irrigated blocks, at various periods during the three summers (Fig. S1.1), may have caused a significant change in the composition of root exudates, both during these drought periods and after rewetting

(Gargallo-Garriga et al. 2018). Alternating periods of drying and rewetting, and changes in root exudation, can also have an influence on microbial hotspots and thus microbial abundance and activity (Kuzyakov and Blagodatskaya 2015), which could help explain lower EEAs in control blocks.

In line with our expectations, the effect of irrigation was higher in the topsoil for the P-related EEA (Fig. 1.5). This is probably because microbial communities benefit more of increasing humidity in soil layers that are more subjected to drought and where the substrate is relatively nutrient rich (Fanin et al. 2020). However, this effect was relatively constant throughout the soil column for N-related EEAs. This result is in line with the idea that there is a shift in the requirements of microbial communities at the community scale along the soil profile (Fanin et al. 2013; Mooshammer et al. 2014), and may explain the variability in nutrient availability from the top to the bottom soil layers (Fig. 1.6). Indeed, irrigation had a negative effect on available N in the topsoil. This is probably due to important N uptake by trees and the understory, coupled with a leaching of soluble mineral N-forms (NH_4^+ and NO_3^-) into the deeper soil layers, where irrigation had a positive effect on available N (Table 1.2, Fig. 1.6A). In contrast, the available P was generally low and unaffected by the different factors (Table 1.2, Fig. 1.6B). The lack of significant increase in available P within the 0–30 cm soil layer may be due to a strong and rapid absorption by tree roots in the topmost layer. High understory root biomass in deeper soils (Table S1.2) and a general decrease in P desorption along the soil profile (Achat et al. 2013b), may explain the decrease in available P along the soil profile. The differing effects of water availability on available nutrients point towards a strong influence of organic matter quality and associated microorganism communities to explain the EEAs' responses.

Furthermore, because C-related enzymes were not as affected as other EEAs by irrigation, the result reinforces the idea that changes in organic matter quality are also important drivers of EEAs under low water availability. The lower litterfall and forest floor C:N ratios in dry blocks compared to irrigated blocks support this hypothesis (Table S1.1). Indeed, higher tree growth rates when water conditions are favourable reduce available N input to soils, with further consequences on N limitations in our study system. Along the entire soil profile, we found less available N in the non-irrigated blocks (Table S1.3). Taken together, these results highlight that changes in precipitation regimes may have important consequences on EEAs through direct effects of water availability and indirect effects through tree growth on organic matter quality and nutrient availability.

1.4.3 Effect of the interaction between mixing tree species and water availability

Although we hypothesized that mixing birch and pine could mitigate the effects of lower water availability on nutrient availability and EEAs, we did not find such a compensatory

effect (Table 1.2). Studies at larger scales have shown that biodiversity may become more beneficial as water limitations increase (Lebourgeois et al. 2013; Pretzsch et al. 2013b; Ratcliffe et al. 2017), but some other studies contradict this statement. For example, Jucker et al. 2014b found that the magnitude of the biodiversity effect on aboveground biomass production decreases in strength during drought years, and Jactel et al. 2018 found high overyielding due to mixing tree species at wet sites at the stand level. Additionally, we suggest that the lack of a significant interaction effect between mixing birch and pine trees and water availability might be due to the spatial segregation of the individual effects of these two factors. While mixing the two tree species tended to increase EEAs in the mid-soil layer, irrigation tended to increase EEAs and decrease available N in the top soil. Moreover, we collected our samples in March, when the control blocks were not under water stress and the irrigation treatment had not yet begun for the year (Table 1.1).

Finally, the lack of effect due to the species mixing may be simply the consequence of the lack of differences between the tree species themselves. Indeed, we did not see different patterns of the effect of water availability on EEAs between the two monocultures. This might be surprising given that *Pinus pinaster* is a fairly more drought tolerant species compared to *Betula pendula* (Choat et al. 2012). However, the tolerance difference is quantitatively modest (sapwood hydraulic vulnerability P50: *Pinus pinaster* = -2.8 to -3.7 Mpa, *Betula pendula* = -2.2 to -2.4 Mpa; Choat et al. 2012), and both species' roots similarly explored the entire soil profile in the dry control and irrigation treatments (Table S1.2), which could explain the small effects of species identity and, in turn, of mixing species. Additional analyses including more tree species and more sampling dates are needed to further evaluate the relationship between EEAs and tree diversity, as well as intra annual temporal variation of EEAs and biodiversity effects (Cardinale et al. 2012).

1.4.4 Environmental effects and larger implications for nutrient cycling

The RDA gives us new insight into how environmental variables may have an effect on different enzyme activities (Fig. 1.7). Available N was negatively correlated with the tree species' fine root biomass along the first axis, likely due to rapid nutrient absorption by roots, which led to N-depletion in the topsoil where roots thrive (Giehl and von Wirén 2014). N-related EEAs may have been stimulated by this low N availability in order to maintain high growth rates (Kaye and Hart 1997), or by tree root exudation (Renella et al. 2006; Zhang et al. 2019). Indeed, N-related EEAs were also positively correlated to tree species' fine root biomass (Fig. 1.7). To a lesser extent, the P-related enzyme was negatively correlated to available P, likely due to similar feedback mechanisms.

Interestingly, we also found that C-related EEAs were strongly related to available P. This implies that when P availability is high, microorganisms are strongly limited by C,

inciting them to produce more C-related enzymes to get access to the energy produced by decomposing organic matter (Fanin et al. 2017; Fanin et al. 2016a). This hypothesis was supported by the significant positive correlation between available P and the C_{enz}:P_{enz} ratio across all species and water availability treatments (Fig. 1.8B), which we did not find between the available N and the C_{enz}:N_{enz} ratio (Fig. 1.8A). This is likely because alleviating P limitation generates a new constraint for microorganisms and thus the necessity to acquire C from organic molecules in these P-poor podzol soils (Achat et al. 2009; Augusto et al. 2017). This relationship was true regardless of species composition, water availability, or soil depth, suggesting that P limitation plays a more important role in controlling C-related enzymes. Thus, potential C sequestration is likely to be limited by P availability in these nutrient-poor forests.

1.4.5 Conclusions

This study revealed that water supply and mixing pine and birch trees could have important long-term effects on soil extracellular enzyme activities (EEAs). First, from a management perspective, our results suggest that overall C sequestration in our system is likely to be constrained by P availability, regardless of water availability. Second, we found C-N-P decoupling effects as a result of tree species mixing or improved water availability, which may alter microorganism stoichiometry and belowground C, N, P fluxes. Finally, the lack of interactive effects between mixing birch and pine, and water supply, suggests that the negative effect of lower water availability on microbial activity cannot be offset by the positive effect of mixing these two species. The next step will be to sample at different seasons to investigate the possible temporal variations of EEAs and to measure other belowground processes such as protein depolymerisation and mineralization, in order to assess the dynamics of interactive effects between water availability and tree diversity over seasons and disentangle underlying mechanisms.

Acknowledgements

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1.5 Supplementary information

Table S1.1: Soil texture in dry control and irrigated blocks. A composite was made from all species composition treatments (pure birch, pure pine, mixed birch + pine) per block. Data are mean ± standard error of the mean (n = the total number of samples per treatment).

	Depth (cm)	n	Clay (g kg^{-1})	Fine silt (g kg^{-1})	Coarse silt (g kg^{-1})	Fine sand (g kg^{-1})	Coarse sand (g kg^{-1})
Control	0–5	4	38.5 ± 2.0	20.3 ± 3.0	4.8 ± 1.5	45.8 ± 4.4	890.8 ± 6.2
	5–15	4	32.3 ± 2.6	19.8 ± 3.1	5.0 ± 1.7	51.5 ± 1.7	891.5 ± 4.4
	15–30	4	36.8 ± 2.5	21.0 ± 3.0	3.8 ± 2.1	46.0 ± 3.3	892.5 ± 2.7
	30–60	4	19.8 ± 0.5	3.3 ± 0.8	2.8 ± 0.9	52.5 ± 6.2	921.8 ± 6.0
	60–90	4	14.8 ± 3.1	3.3 ± 1.6	2.8 ± 1.8	55.5 ± 15.2	923.8 ± 21.6
Irrigation	0–5	4	36.3 ± 1.3	22.8 ± 3.5	6.5 ± 0.6	52.0 ± 4.4	882.5 ± 8.1
	5–15	4	38.8 ± 3.4	30.0 ± 4.7	8.0 ± 1.4	55.5 ± 2.9	867.8 ± 7.9
	15–30	4	44.3 ± 6.8	28.8 ± 4.2	7.5 ± 1.5	52.0 ± 4.7	867.5 ± 11.2
	30–60	4	18.3 ± 0.9	6.0 ± 1.2	2.8 ± 1.1	52.8 ± 8.4	920.3 ± 10.2
	60–90	4	14.0 ± 0.9	3.3 ± 0.9	0.5 ± 0.3	50.3 ± 13.6	932.0 ± 13.9

Table S1.2: Soil moisture, soil pH, tree fine root biomass (FRB), and understory FRB along the soil profile in the three different species composition treatments (pure birch, pure pine, mixed birch + pine), each in both dry control and irrigated blocks. Data are mean ± standard error of the mean (n = the total number of samples per treatment).

	Depth (cm)	n	Birch		Birch + Pine		Pine	
			Control	Irrigation	Control	Irrigation	Control	Irrigation
Litterfall biomass ($g\ m^{-2}\ yr^{-1}$)	-	6	50.77 ± 12.31	78.55 ± 25.14	104.0 ± 35.53	111.4 ± 38.42	171.2 ± 65.71	151.6 ± 58.29
Soil moisture (%)	0–5	16	15.77 ± 1.00	15.72 ± 1.08	13.10 ± 1.04	14.82 ± 0.74	12.30 ± 1.07	17.89 ± 1.36
	5–15	16	14.33 ± 1.07	11.38 ± 1.12	14.72 ± 1.56	14.06 ± 1.85	13.62 ± 2.05	13.43 ± 1.23
	15–30	16	13.68 ± 0.95	12.37 ± 0.66	11.82 ± 0.49	10.98 ± 0.80	13.50 ± 0.56	12.39 ± 0.80
	30–60	16	11.07 ± 0.62	12.01 ± 0.82	10.28 ± 0.73	13.61 ± 1.51	10.58 ± 0.85	14.21 ± 0.99
	60–90	16	13.41 ± 0.44	13.52 ± 0.67	13.00 ± 0.36	12.47 ± 0.44	12.79 ± 0.62	13.28 ± 0.44
Soil pH [H_2O]	0–5	4	4.53 ± 0.02	4.64 ± 0.07	4.57 ± 0.10	4.44 ± 0.05	4.48 ± 0.11	4.61 ± 0.22
	5–15	4	4.27 ± 0.03	4.43 ± 0.03	4.18 ± 0.05	4.20 ± 0.06	4.29 ± 0.09	4.50 ± 0.26
	15–30	4	4.27 ± 0.07	4.38 ± 0.12	4.03 ± 0.06	4.11 ± 0.04	4.27 ± 0.07	4.47 ± 0.20
	30–60	4	4.54 ± 0.07	4.68 ± 0.06	4.57 ± 0.05	4.59 ± 0.09	4.64 ± 0.02	4.79 ± 0.13
	60–90	4	4.77 ± 0.06	4.85 ± 0.05	4.71 ± 0.03	4.71 ± 0.04	4.69 ± 0.04	4.84 ± 0.11
Tree FRB ($g\ m^{-3}$)	0–5	16	1099.74 ± 211.82	1696.49 ± 253.16	988.80 ± 162.96	1289.18 ± 129.3	850.63 ± 169.80	752.70 ± 117.46
	5–15	16	611.62 ± 95.13	809.71 ± 119.78	637.58 ± 68.08	597.93 ± 71.83	777.70 ± 135.93	432.76 ± 43.63
	15–30	16	139.49 ± 38.10	408.30 ± 119.35	254.78 ± 58.01	262.84 ± 60.62	208.53 ± 61.23	304.15 ± 83.03
	30–60	16	64.31 ± 24.27	174.56 ± 47.1 1	136.71 ± 44.40	189.36 ± 70.03	109.02 ± 56.76	92.66 ± 31.59
	60–90	16	42.44 ± 36.97	51.96 ± 33.25	5.37 ± 3.51	13.56 ± 13.56	5.80 ± 4.45	8.46 ± 5.79
Understory FRB ($g\ m^{-3}$)	0–5	16	707.77 ± 136.57	660.29 ± 135.92	348.72 ± 55.36	710.20 ± 207.54	598.50 ± 92.95	645.67 ± 130.35
	5–15	16	1292.80 ± 239.27	861.00 ± 118.49	1159.27 ± 173.34	1292.09 ± 210.32	911.06 ± 140.76	1141.48 ± 231.64
	15–30	16	591.49 ± 93.85	791.66 ± 133.58	868.49 ± 122.23	781.45 ± 147.10	992.93 ± 139.29	656.93 ± 119.26
	30–60	16	418.74 ± 98.36	429.95 ± 96.06	372.79 ± 72.74	511.32 ± 100.01	577.75 ± 79.59	479.34 ± 124.09
	60–90	16	140.12 ± 92.41	12.70 ± 10.14	69.13 ± 37.59	15.17 ± 7.97	42.01 ± 19.01	80.27 ± 45.82

Table S1.3: Selected variables averaged within dry control and irrigation blocks (averaged between species composition treatments) for each soil layer. Data are mean ± standard error of the mean (n = the total number of samples per treatment) at each layer, and a weighted mean per treatment.

	Depth (cm)	n	Control	n	Irrigation
C-related EEAs (mmol kg^{-1})	0-5	48	0.804 ± 0.043	47	1.02 ± 0.060
	5-15	48	0.705 ± 0.047	46	0.862 ± 0.050
	15-30	48	0.795 ± 0.075	47	0.844 ± 0.079
	30-60	48	0.254 ± 0.024	47	0.284 ± 0.026
	60-90	48	0.049 ± 0.009	47	0.067 ± 0.011
	weighted mean	48	0.436 ± 0.018	46	0.496 ± 0.024
N-related EEAs (mmol kg^{-1})	0-5	47	0.193 ± 0.014	47	0.259 ± 0.016
	5-15	48	0.128 ± 0.011	47	0.159 ± 0.012
	15-30	47	0.127 ± 0.013	47	0.163 ± 0.016
	30-60	48	0.065 ± 0.005	47	0.872 ± 0.011
	60-90	48	0.020 ± 0.002	47	0.028 ± 0.006
	weighted mean	46	0.090 ± 0.005	47	0.115 ± 0.006
P-related EEA (mmol kg^{-1})	0-5	48	0.520 ± 0.025	47	0.659 ± 0.041
	5-15	48	0.381 ± 0.038	47	0.442 ± 0.036
	15-30	48	0.347 ± 0.033	47	0.411 ± 0.032
	30-60	48	0.189 ± 0.017	47	0.200 ± 0.018
	60-90	48	0.055 ± 0.009	47	0.064 ± 0.007
	weighted mean	48	0.261 ± 0.017	47	0.296 ± 0.018
Available N (µg N g^{-1})	0-5	48	0.943 ± 0.219	48	0.229 ± 0.059
	5-15	48	1.503 ± 0.165	47	1.080 ± 0.163
	15-30	47	1.859 ± 0.219	48	1.850 ± 0.289
	30-60	48	2.071 ± 0.212	48	2.790 ± 0.411
	60-90	48	1.181 ± 0.182	46	2.261 ± 0.469
	weighted mean	47	1.467 ± 0.125	45	1.700 ± 0.224
Available P (µg P g^{-1})	0-5	48	1.740 ± 0.213	46	2.038 ± 0.163
	5-15	47	2.874 ± 0.250	48	3.400 ± 0.315
	15-30	47	2.568 ± 0.239	48	3.066 ± 0.291
	30-60	47	1.710 ± 0.245	48	1.911 ± 0.231
	60-90	48	0.645 ± 0.162	47	0.638 ± 0.113
	weighted mean	46	1.793 ± 0.142	45	2.000 ± 0.150

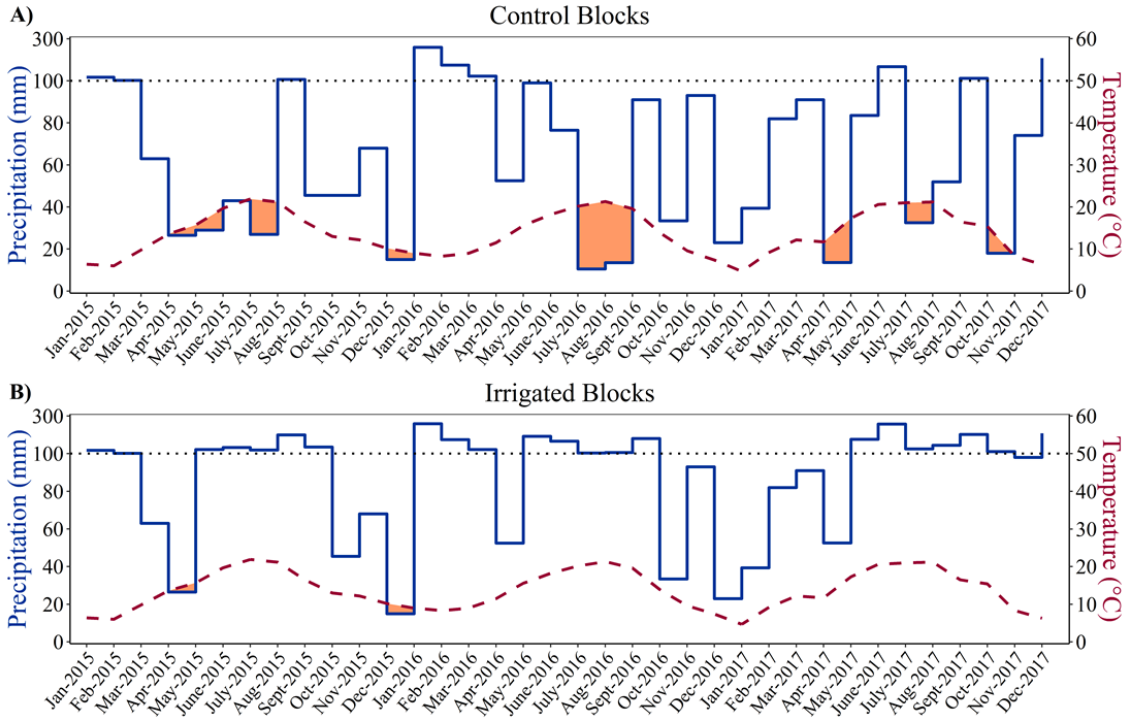

Figure S1.1: Ombrothermic diagram with total monthly precipitations (blue solid line) and average temperatures (red dashed line) from Jan. 2015- Dec. 2017 in A) control blocks and B) irrigated blocks, which includes the additional precipitation (3 mm per night) when irrigation is active (on average early May to late September). Temperature scale is set to 2mm/°C, and values above the 100mm black line are scaled to 20mm/°C (Walter and Lieth 1967). When temperature is two times greater than precipitation, there is an arid period (orange). Data are from the local INRAE weather station at Cestas Pierroton (44.742 °N - 0.782 °E), less than 2km from the experimental site.

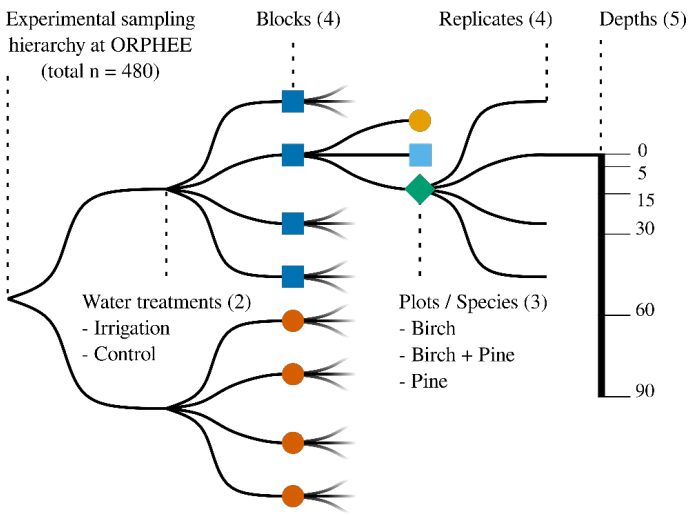

Figure S1.2: Sampling hierarchy for soil extracellular enzyme activities, available nutrients, tree fine root and understory fine root biomass. Irrigation is randomly assigned to different blocks and not spatially grouped.

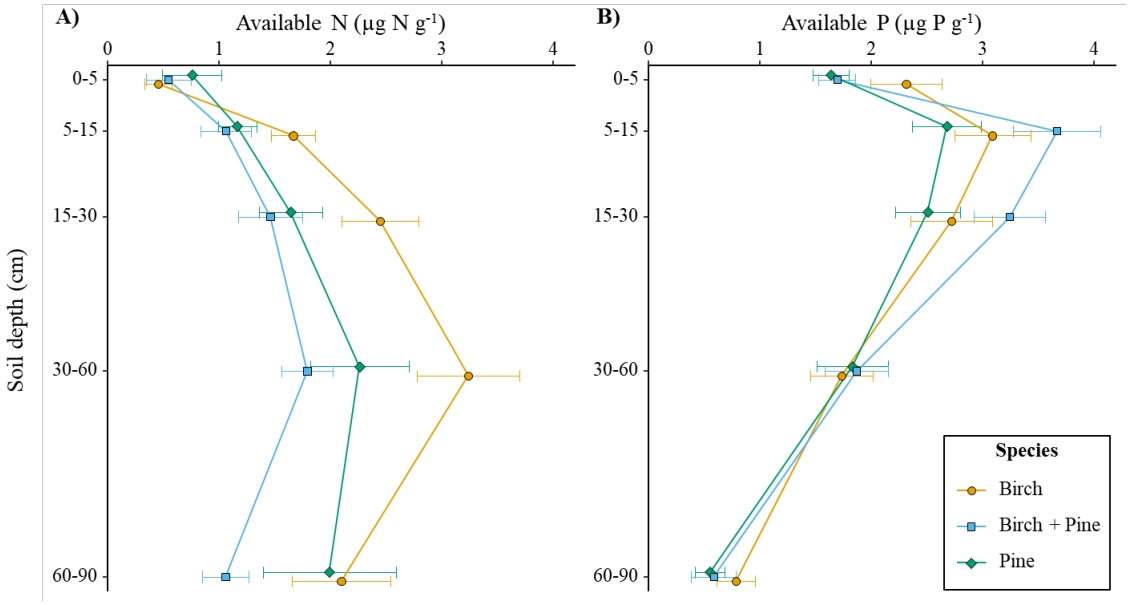

Figure S1.3: Distribution of the available nutrients along the soil profile in pure birch (circles, *yellow*), mixed birch + pine (squares, *blue*) and pure pine plots (diamonds, *green*); A) available N (µg N g^{-1}); and B) available P (µg P g^{-1}). The water availability treatments were pooled for each species composition treatment. Data points represent means at each depth interval of the four replicates per plot, in each of the eight blocks (n = 32) and error bars represent standard errors of the mean.

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CHAPTER TWO

High water availability stimulates soil organic nitrogen breakdown, while tree mixing has little effect

Manuscript soon-to-be submitted by Maxwell, T.L., Augusto, L., Tian, Y., Wanek, W., & Fanin, N.

Abstract

To increase and stabilize forest productivity in a changing climate, mixing tree species has been increasingly studied as a long-term management strategy. Yet, little is known about soil organic nitrogen breakdown (i.e. the bottleneck of belowground nutrient cycling) in mixed species forests, and even less under low water availability. In this study, we used a diversity experiment in southwestern France (subjected to frequent water shortages in summer) to assess the interactive effects of water availability (*via* irrigation) and mixing tree species (monocultures of *Pinus pinaster*, *Betula pendula* versus mixed plots of pine-birch). We measured gross soil organic nitrogen breakdown rates using a novel high-throughput isotope pool dilution method, along with microbial biomass to calculate specific activity. Overall, high water availability led to an increase in organic N breakdown compared to the control plots, which may be due to an increase in substrate availability, but we found no effect of species composition on breakdown rates. When investigating the interactive effect of mixing and water availability, results suggest that mixing tree species had a negative effect on soil organic nitrogen breakdown in the control blocks subject to dry summers, but that this effect tended to become positive with high water availability. These results suggest that water availability has the potential to influence potential mixing effects on soil organic N cycling in forests.

Keywords: biodiversity, microbial biomass, precipitation, protein depolymerization, TreeDivNet

Résumé

Dans ce chapitre, nous avons étudié l'interaction entre le mélange des espèces d'arbres et la ressource en eau sur la dégradation de l'azote organique du sol. Pour cela, nous avons échantillonné du sol (0-15 cm) au site d'expérimentation ORPHEE, au sud-ouest de la France et mesuré ce processus en utilisant une nouvelle méthode de dilution isotopique, ainsi que la biomasse microbienne *via* fumigation-extraction. Une forte disponibilité en eau a entraîné une augmentation des taux de dégradation d'azote organique, ce qui peut être dû à une augmentation de la disponibilité du substrat. En étudiant l'effet interactif, les résultats suggèrent que le mélange des espèces d'arbres a eu un effet négatif sur la dégradation de l'azote organique du sol dans les blocs soumis à des étés secs, mais que cet effet a eu tendance à devenir positif avec une disponibilité élevée en eau.

Mots clés: biodiversité, biomasse microbienne, dépolymérisation des protéines, précipitation, TreeDivNet

Chapter Introduction

Soil organic nitrogen breakdown

General context

Because over 50% of total soil nitrogen (N) is represented by peptidic structures (Schulten and Schnitzer 1998) and contributes approximately 90% of total N in plant litter and microbial residues (Jan et al. 2009), depolymerization of proteins to oligopeptides and free amino acids (i.e. the organic nitrogen breakdown prior to mineralization, Fig. 2.1), drives the soil N cycle and determines the amount of N available to plants and microbes (Jan et al. 2009; Jones et al. 2002). Few studies have examined gross rates of protein depolymerization, a process key to belowground N cycling, and none have looked into how mixing tree species may affect this breakdown. Additionally, we wanted to investigate how differences in water availability may impact potential effects of mixing tree species.

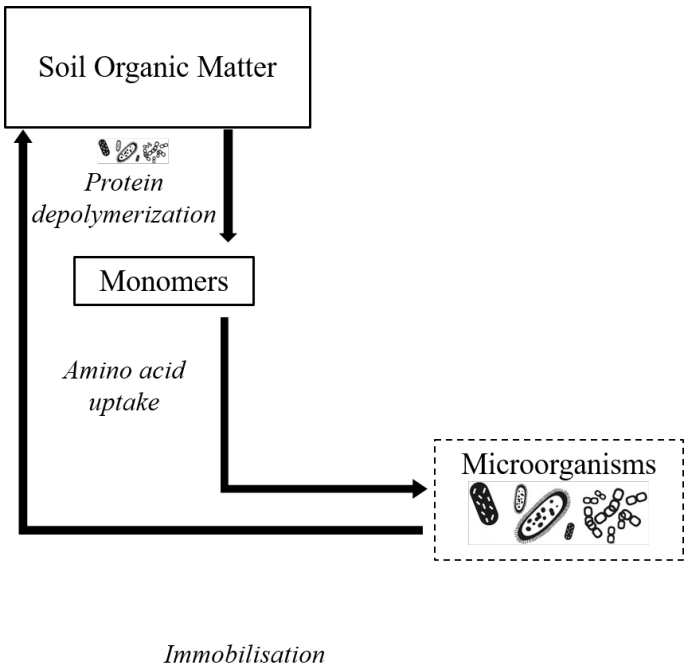

Figure 2.1: Soil organic nitrogen breakdown (protein depolymerization) is the bottleneck of belowground N cycling.

Objective and hypotheses

In this study, we tested the interactive effect of mixing tree species and water availability on soil organic N breakdown.

We hypothesized ($H_{2.1}$) that mixing tree species would increase soil organic N breakdown rates, since mixing different litter qualities may lead to more accelerated litter decomposition (Chapman et al. 2013; Cornwell et al. 2008) and an increase in microbial biomass (Chen et al. 2019a; Khlifa et al. 2017; Lucas-Borja et al. 2012; Thakur et al. 2015).

Secondly, we hypothesized ($H_{2.2}$) that an increase in water availability would accelerate the breakdown due to its stimulation of microbial activity (Brockett et al. 2012).

Finally, we hypothesized ($H_{2.3}$) that the positive effect of mixing would be strongest in the plots subject to the dry summers of southwestern France, due to facilitation mechanisms between microorganisms to decompose litter with differing quality, which were found to be more present under limited water conditions (Ratcliffe et al. 2017).

Experimental design

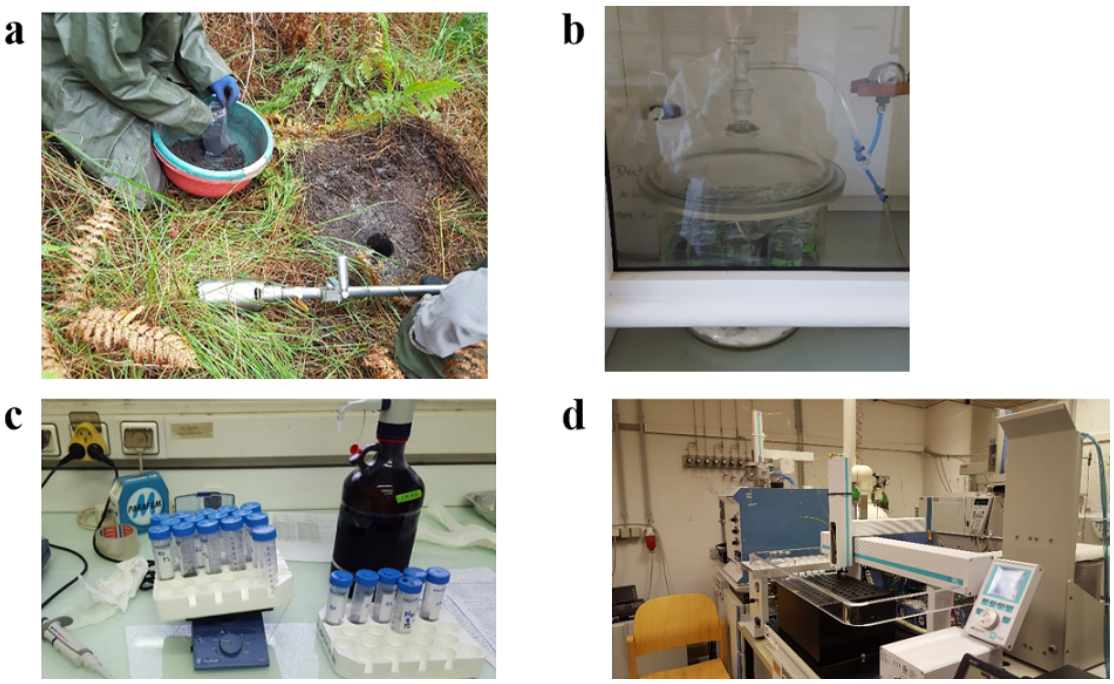

Figure 2.2: a) Soil sampling using a corer and homogenizing the soil, b) fumigation for microbial biomass measurements, c) agitation post-isotope labeling of amino acids, d) measurement of ^{15}N-AA on a PT-IRMS.

In July 2020, we collected three soil cores (0-15 cm layer) per plot at the ORPHEE field site (birch, pine, birch + pine), each in 3 replicated blocks of low and high water availability. Microbial biomass carbon (C) and N were measured on fresh soil samples using the fumigation-extraction method (Vance et al. 1987). To study the key organic nitrogen cycling breakdown rates (protein depolymerization and amino acid uptake by microorganisms), we used the isotope pool dilution technique (Wanek et al. 2010) and a novel high-throughput method (Noll et al. 2019a, Fig. 2.2).

Main results

Overall, we did not find a significant effect of mixing birch and pine on protein depolymerization, nor on the microbial biomass, contrary to our hypothesis ($H_{2.1}$). However, high water availability led to an increase in protein depolymerization, without a significant effect on microbial biomass N or on specific activity, partially validating hypothesis ($H_{2.2}$). This suggests that the positive effect of high water availability on protein depolymerization was mainly due to an increase in substrate solubility and/or an increase in the encounter between substrates and enzymes, due to both a higher chance for spatial displacement and facilitated movement under high water availability.

While we did not find significant interactive effects between mixing tree species and water availability on soil organic N breakdown, trends emerged opposite to our expectation ($H_{2.3}$). These results suggest that mixing tree species had a negative effect on protein depolymerization in the control blocks subject to dry summers, but that this effect tended to become positive with high water availability. These results suggest that water availability may influence potential mixing effects on soil organic N cycling in forests.

Take-home message

Mixing tree species tended to increase protein depolymerization rates under high water availability, due to both of these factors increasing substrate and water availability for soil microorganisms. Overall, water availability was the strongest driver, increasing soil organic N breakdown rates in all species compositions plots.

2 Paper 2: High water availability stimulates soil organic nitrogen breakdown, while tree mixing has little effect

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Mixed birch and pine trees at ORPHEE on June 3rd 2020. Photo: T. L. Maxwell.

2.1 Introduction

Forests provide a multitude of ecosystem services (Gamfeldt et al. 2013; Jactel et al. 2017; Liang et al. 2016), yet they are increasingly threatened by more intense and frequent drought periods (IPCC 2014; Schwalm et al. 2012), with important negative consequences on tree productivity and survival (Hartmann et al. 2018). Mixing tree species is a well-explored option to mitigate these negative effects (Lebourgeois et al. 2013; Pretzsch et al. 2013b), as mixed-species forests may be more stable to climatic fluctuations due to species asynchrony and favorable species interactions (Jucker et al. 2014a; Kardol et al. 2018; Morin et al. 2014), which may lead to an increased productivity compared to pure stands (Baeten et al. 2019; Cardinale et al. 2007; Liang et al. 2016; Zhang et al. 2012). However, little is known how water availability may affect mixing effects on belowground nutrient cycling (Richards et al. 2010).

Nitrogen (N) is one of the most important macronutrients as it constrains growth and activity of all living organisms. Because over 50% of total soil N is represented by peptidic structures (Schulten and Schnitzer 1998) and contributes approximately 90% of total N in plant litter and microbial residues (Jan et al. 2009), depolymerization of proteins to oligopeptides and free amino acids (i.e. the organic nitrogen breakdown prior to mineralization) drives the soil N cycle and determines the amount of N available to plants and microbes (Jan et al. 2009; Jones et al. 2002). The emergence of isotope techniques has allowed for the quantification of gross protein depolymerization (Kirkham and Bartholomew 1954; Wanek et al. 2010), which has been improved with a recently published high throughput approach (Noll et al. 2019a). The studies using these methods have evidenced that organic nitrogen breakdown responds differently to various global changes, such as drought, warming, and elevated CO_2 (Andresen et al. 2015; Fuchslueger et al. 2019; Maxwell et al. accepted; Wild et al. 2018). More importantly, several studies in a variety of ecosystem and soil types, found that protein depolymerization is a substrate-limited rather than enzyme-limited process (Maxwell et al. accepted; Noll et al. 2019b; Wanek et al. 2010), and that it is stoichiometrically regulated, i.e. dependent on litter C:nutrient ratios (Mooshammer et al. 2012). However, we do not know how mixing tree species may affect organic N breakdown, and whether this effect can be impacted by changes in water availability.

In this study, we used a 12-year-old experimental forest that manipulates both tree diversity and water availability (*via* irrigation), to test the interactive effect of mixing tree species and water availability on soil organic N breakdown. We hypothesized (H_1) that mixing tree species would lead to an increase in soil organic N breakdown rates since mixing different litter qualities may lead to faster litter decomposition (Chapman et al. 2013; Cornwell et al. 2008) and an increase in microbial biomass (Chen et al. 2019a; Khlifa et al. 2017;

Lucas-Borja et al. 2012; Thakur et al. 2015). Secondly, we hypothesized (H_2) that an increase in water availability would accelerate the breakdown due to its stimulation of microbial activity (Brockett et al. 2012). Finally, we hypothesized (H_3) that the positive effect of mixing would be strongest in the plots subject to the dry summers of southwestern France, due to facilitation mechanisms between microorganisms to decompose litter with differing quality, which were found to be more present under limited water conditions (Ratcliffe et al. 2017).

2.2 Methods

We conducted this study at the ORPHEE experimental site in southwestern France, 40 km southwest of Bordeaux in the *Landes de Gascogne* region (44°44.35'N - 00°47.9'W; see Supporting Information for more details). Briefly, the ORPHEE experiment is composed of several species combinations randomly located within 6 equally distributed blocks (3 control blocks and 3 irrigated blocks for low availability and high availability, respectively). Irrigation was initiated in 2015 and has been applied seasonally (3 mm per day from May to October) at the block level to alleviate water shortage during the growing season, when summers are dry. To assess the effects of mixing tree species, we selected two monocultures (*Betula pendula*, 'birch', and *Pinus pinaster*, 'pine'), and their mixture (birch + pine). Each plot was composed of 100 trees within a 20 × 20 m area.

In July 2020 (five years after irrigation started), we collected three soil cores per plot using a soil corer of 8 cm diameter and 15 cm depth. The Xylosylve monitoring platform nearby (1.5 km away) indicated that the area received only 2 mm of precipitation during the four weeks prior to sampling. Each soil sample was sieved to 2 mm, homogenized, and stored at 4°C until further analyses. Aliquots of fresh soil were weighed and dried (50°C, at least 72 h) to calculate the fresh weight to dry weight ratio and soil moisture. Microbial biomass carbon (C) and nitrogen (N) were measured on an aliquot of the fresh soil sample using the fumigation-extraction method (Vance et al. 1987). Measured N in the extracts from non-fumigated soils was used to estimate 'extractable N'.

To study the key organic nitrogen cycling breakdown rates (protein depolymerization [PD] and amino acid uptake by microorganisms [AAU]), we used the isotope pool dilution technique (Wanek et al. 2010) and a novel high-throughput method (Noll et al. 2019a). The total free amino acid [TFAA] concentrations in the extracts were measured by fluorometric determination (Jones et al. 2002), and corrected for ammonium fluorescence, measured beforehand by colorimetric determination (Hood-Nowotny et al. 2010). Knowing the TFAA in the extracts and soil moisture in the soils, two different tracer concentrations and volumes were calculated (one for each water availability treatment) so that around 60% water holding capacity was obtained with a ^{15}N-AA concentration (98‰, Cambridge

Isotopes) that was no larger than 20% of the original amino acid pool. The isotope pool dilutions were done on two aliquots of fresh soil, one stopped after 15 min and the other 45 min after tracer addition, after which soils were extracted with cold 1M KCl. Ammonium was removed from the extracts by microdiffusion. Free amino acids in the ammonium-free extracts were then converted to N_2O gas which was analyzed by purge-and-trap isotope ratio mass spectrometry for the isotopic composition and concentration of the amino-acids (Lachouani et al. 2010; Noll et al. 2019a). Using the change of ^{15}N-^{14}N amino acids over time, organic nitrogen breakdown rates were calculated (Kirkham and Bartholomew 1954).

All of the statistical analyses were done using R software (version 4.1.0). First, extreme values were removed following the outlier labelling rule with a conservative tuning parameter of g = 2.2 (Hoaglin and Iglewicz 1987). Mixed models were used to assess the effect of the following fixed effect factors: tree species composition (pure birch, pure pine, mixed birch-pine), water availability (low, high) and their interaction on soil organic N breakdown rates, microbial biomass, and specific activity. Pairs of low and high H_2O blocks (i.e. one 'Superblock') were included as a random factor to account for the spatial structure of our experimental design, with tree species composition for each H_2O treatment nested into 'Superblock'. We then applied contrasts and performed post-hoc tests Tukey HSD post-hoc tests, with $\alpha = 0.05$ used as the level to define significance.

Table 2.1: Results of mixed model analysis of species composition (Sp), water availability (H_2O) and their interaction on soil nutrient availability, productivity, and nutrient relations at the IDENT-SSM and ORPHEE sites. F-values and *p*-values for fixed effects are presented. Significant ($p < 0.05$) effects are noted in bold font.

	Species (Sp)		H_2O		Sp x H_2O	
	F-value	p-value	F-value	p-value	F-value	p-value
Protein depolymerization (PD)	0.015	0.985	8.483	**0.015**	2.387	0.142
Amino acid uptake (AAU)	0.201	0.821	9.424	**0.012**	2.213	0.160
Microbial biomass C	0.484	0.630	0.479	0.505	0.58	0.578
Microbial biomass N	0.053	0.949	0.285	0.605	0.656	0.540
Specific PD activity	0.249	0.785	0.719	0.416	0.041	0.960
Specific AAU activity	0.35	0.713	0.643	0.441	0.134	0.876

2.3 Results and discussion

Overall, the free amino acid content (Fig. S2.1) averaged around 2.95 ± 0.24 µg N g^{-1} (mean ± s.e.), gross protein depolymerization between 6.51 and 95.99 µg N g^{-1} d^{-1} and amino acid uptake between 6.09 and 117.26 µg N g^{-1} d^{-1} (Fig. 2.3). These values are within the range of those found in previous studies (Fuchslueger et al. 2019; Noll et al. 2019b), but on the low side due to the nutrient poverty of the sandy soil at our field site (Achat et al. 2009; Augusto et al. 2010).

In contrast to our first hypothesis (H_1), no overall species composition effect on gross protein depolymerization or amino acid uptake emerged (Table 2.1, Fig. 2.3). Previously, protein depolymerization has been found to be a substrate-limited rather than by extracellular enzyme content (Maxwell et al. accepted; Noll et al. 2019b). Because soil enzyme activity did not differ between species composition treatments in the top 0-15 cm soil layer at our site (Maxwell et al. 2020a), these results suggest that the variability and the trends of organic N breakdown rates are thus due to differences in substrate availability (i.e. soil organic nitrogen, or proteins) within and between species composition treatments.

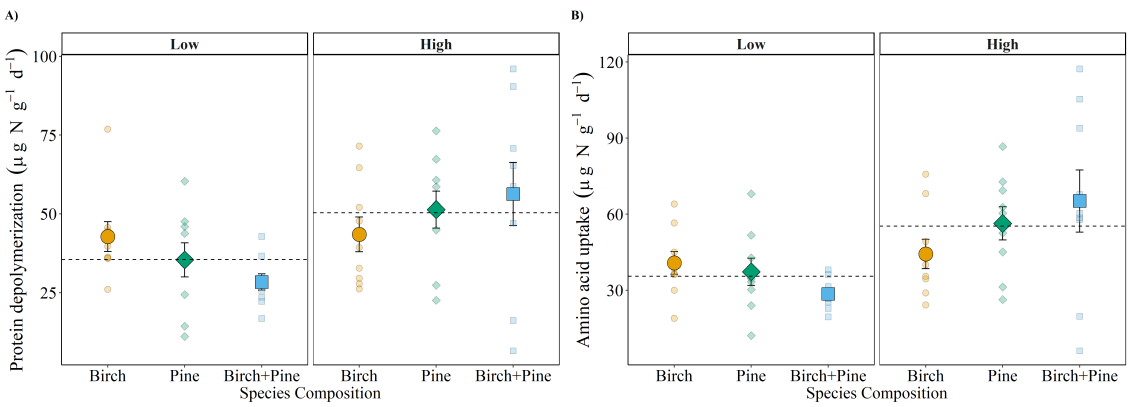

Figure 2.3: Effect of tree species composition and water availability (low *vs.* high) on A) protein depolymerization [PD] and B) amino acid uptake [AAU]. Raw data are presented in partial translucence, with the mean and standard error superposed (n = 9). Dashed lines correspond to the mean for each water availability treatment.

In agreement with our second hypothesis (H_2), we found a slight positive effect of water availability on both protein depolymerization and amino acid uptake (Table 2.1, Fig. 2.3). At this sampling period in July during peak summer dry conditions, soil moisture differed between control (5.70 ± 0.50%, mean ± s.e.) and irrigated blocks (10.49 ± 0.81%). This result indicates that N processing by microbial communities is strongly dependent on soil moisture, probably because microorganisms have a greater N demand under favorable water conditions (Cui et al. 2019; Tiemann and Billings 2011). However, the lack of effect on microbial biomass N or specific activity (Fig. S2.1) suggests that the effect of high water availability on protein depolymerization was mainly due to an increase in substrate solubility and/or in the encounter between substrates and enzymes. In line with this idea,

the difference in soil moisture between low and high water availability also significantly affected the correlation between extractable N and protein depolymerization (Fig. 2.4): the relationship was positive under high water availability ($\rho = 0.69$, $p < 0.001$), whereas there was a negative trend under conditions of low water availability ($\rho = -0.30$, $p = 0.128$). This reinforces the idea that protein depolymerization depends directly on substrate accessibility in the soil solution, a hypothesis that was further supported by the positive relationship between protein depolymerization and soil moisture for birch and birch + pine in control plots (Fig. S2.2).

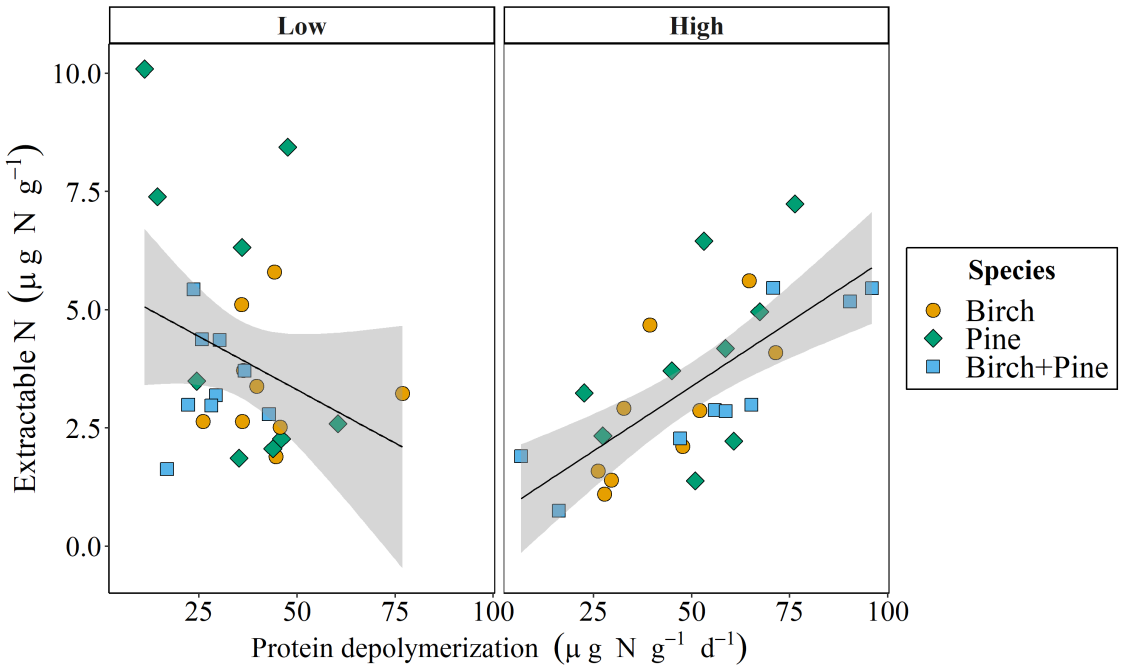

Figure 2.4: Relationship between protein depolymerization and extractable nitrogen [N] for the different species compositions (pure birch, pure pine, mixed birch + pine) in each water availability treatment (low *vs.* high).

Finally, and contrary to our third hypothesis (H_3), we did not find any significant interactive effect of mixing and water availability on soil organic N breakdown (Table 2.1, Fig. 2.3). However, trends did seem to emerge: in control blocks, mixing birch and pine tended to have a negative effect on both protein depolymerization and amino acid uptake, while this mixing under high water availability had a positive effect on these organic N breakdown rates (Fig. 2.3). Because mixing pine and birch has been found to increase the litterfall mass flux (Maxwell et al. submitted), and thus increase substrate availability, an increase in soil moisture in irrigated plots could have led to an increase in protein depolymerization with tree mixing. In contrast, the low water availability in control plots did not allow microbial communities to exploit these additional resources in mixed plots. Instead, the greater biomass of understory vegetation layer in birch plots (Chapter 3, Table 3.3) increased soil moisture (Fig. S2.4), which in turn may have had a positive effect on protein depolymerization rates in these plots. Altogether, these results suggest that water

availability may influence potential mixing effects on soil organic N cycling in forests.

2.4 Conclusion

By using a 12-year-old experimental plantation in southwestern France and a novel high through-put isotope dilution technique, we found that mixing tree species tended to increase protein depolymerization rates under high water availability, due to both of these factors increasing substrate availability for soil microorganisms. In general, water availability was the strongest driver, increasing these soil organic N breakdown rates in all species compositions plots. This was likely due to higher substrate availability in the high water availability plots, but also by connecting the soil microorganisms to their organic matter substrates. While we presented new information regarding potential mixing effects on belowground organic nitrogen cycling, we propose the following to have a better understanding of these mechanisms. First, sampling at different soil layers may provide interesting patterns, as previous studies have found synergistic mixing effects at the mid-soil depth on both soil organic C and on soil enzyme activities (Fanin et al. 2021; Maxwell et al. 2020a) while our study focused on topsoil. Second, sampling at various time points may elucidate potential temporal asynchrony between species in the mixed forest at key phenological time points, such as budburst and fall leaf abscission, in addition to the period of peak leaf phenology that we studied. Finally, combining gross organic N breakdown rate measurements with gross inorganic breakdown rates may also reveal to which degree protein depolymerization is a bottleneck for nitrogen cycling in soils (Noll et al. 2019b; Schimel and Bennett 2004).

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2.5 Supplementary information

2.5.1 Site description

The ORPHEE experiment is located in southwestern France (44°44.35' N, 00°47.9' W, elevation of 60 m asl), 40 km southwest of Bordeaux (Castagneyrol et al. 2013). The mean annual temperature is 12.5°C and the mean annual precipitation is 870 mm (1993 - 2018). Most precipitation occurs from the fall to the spring, leading to climatic moisture deficits during the summer months. The predominant soil type is podzol, characterized by a rapid drainage, coarse texture (95% sand) and low fertility (Augusto et al. 2010), particularly in P (Trichet et al. 2009). The ORPHEE study was established in 2008, after clear-cutting of a *Pinus pinaster* stand, followed by a single ploughing and fertilizer application of 52.4 kg P ha^{-1} and 99.6 kg K ha^{-1}. A total of 256 plots were then planted, with five species (*Betula pendula* Roth. (Be), *Pinus pinaster* Ait. (Pp), *Quercus ilex* L., *Quercus pyrenaica* Willd., *Quercus robur* L. (Qo) in all possible combinations of one to five species mixtures. Each plot has 100 trees planted at 2 m spacing within a 20 m by 20 m plot area. The plots are separated by 3 m from one another, and randomly located within six blocks: three unirrigated, control blocks, subject to the dry summers, and three irrigated blocks. We selected three levels of forest composition to examine tree diversity effects: pure silver birch (Be), pure maritime pine (Pp), and birch-pine mixture (Be-Pp). Plantations containing pine and birch were selected for the study because, contrary to the other tree species, they grew rapidly despite the low fertility of the sandy soil, resulting in relatively dense plots. Seasonal irrigation treatment to alleviate summer plant water stress from early May to late October began in 2015. A two-meter-tall sprinkler installed in the center of each experimental plot delivers 3 mm every evening (equivalent to 21 mm per week) during this period. In the 2019 water treatment months (May 9th – October 28th), for example, control blocks received 396 mm of precipitation, while irrigated blocks received a total of 912 mm of water (precipitation + irrigation) during the same period.

2.5.2 Analytical methods

To study the key organic nitrogen cycling breakdown rates (protein depolymerization (PD) and amino acid uptake by microorganisms (AAU)), we used a novel high-throughput method (Noll et al. 2019a). A 4 g aliquot of fresh soil was extracted using 20 mL of 1M KCl. The total free amino acid (TFAA) concentrations in these extracts were calculated in order to calculate maximum tracer addition rates for the isotope pool dilution (IPD) assays of PD and AAU. The TFAA are measured by fluorimetric determination: a mix of o-phthaldialdehyde and 3-mercaptopropionic acid (OPAME) is added to the samples, yielding a fluorigenic product that is measured at an excitation wavelength of 340 nm and

an emission wavelength of 450 nm (Jones et al. 2002). In order to correct for ammonium fluorescence, its concentration was measured in the same soil extracts by colorimetric determination (Hood-Nowotny et al. 2010). By using an amino-acid mix (AS18, Sigma) for the calibration curve, amino acids in the soil extracts were calculated. Two different tracer concentrations and volumes were calculated (one for each water availability treatment) so that 60% water holding capacity was obtained with a ^{15}N-AA concentration (98‰, Cambridge Isotopes) that was no larger than 20% of the original amino acid pool. The resultant calibration curves, the sample's measured fluorescence, original soil fresh weights, and the fresh to dry weight ratios, soil ammonium and amino acid concentrations were calculated.

In the ^{15}N-IPD assays to measure gross PD and AAU organic nitrogen breakdown rates, the target pool (free amino acids) is enriched by ^{15}N-labeled amino acid mix and the concentrations and ^{15}N-^{14}N ratios of amino acids are measured over time. The influx of unlabeled amino acids from PD and the efflux of tracer from this pool by AAU cause a dilution of the ^{15}N-^{14}N ratio in amino acids and the flux rates can thereby be determined. The isotope pool dilutions were done on two aliquots of 4g of fresh soil, one stopped after 15min and the other 45min after tracer addition, after which soils were extracted with 20 mL cold (4°C) 1M KCl for 30 min at 4°C on a roller (around 60 rpm). Extracts were frozen at -20°C until subsequent analyses. Prior to quantifying the amino acids after the labeling, ammonium had to be removed from the extracts by microdiffusion as ammonium interferes with the conversion of α-amino groups ($-NH_2$) of amino acids to nitrite. Free amino acids in the ammonium-free extracts were then converted to N_2O gas which was analyzed by purge-and-trap isotope ratio mass spectrometry for the isotopic composition and concentration of the amino-acids (Lachouani et al. 2010). Using the change of ^{15}N-^{14}N amino acids over time, organic nitrogen breakdown rates were calculated (Kirkham and Bartholomew 1954).

2.5.3 Supplementary figures

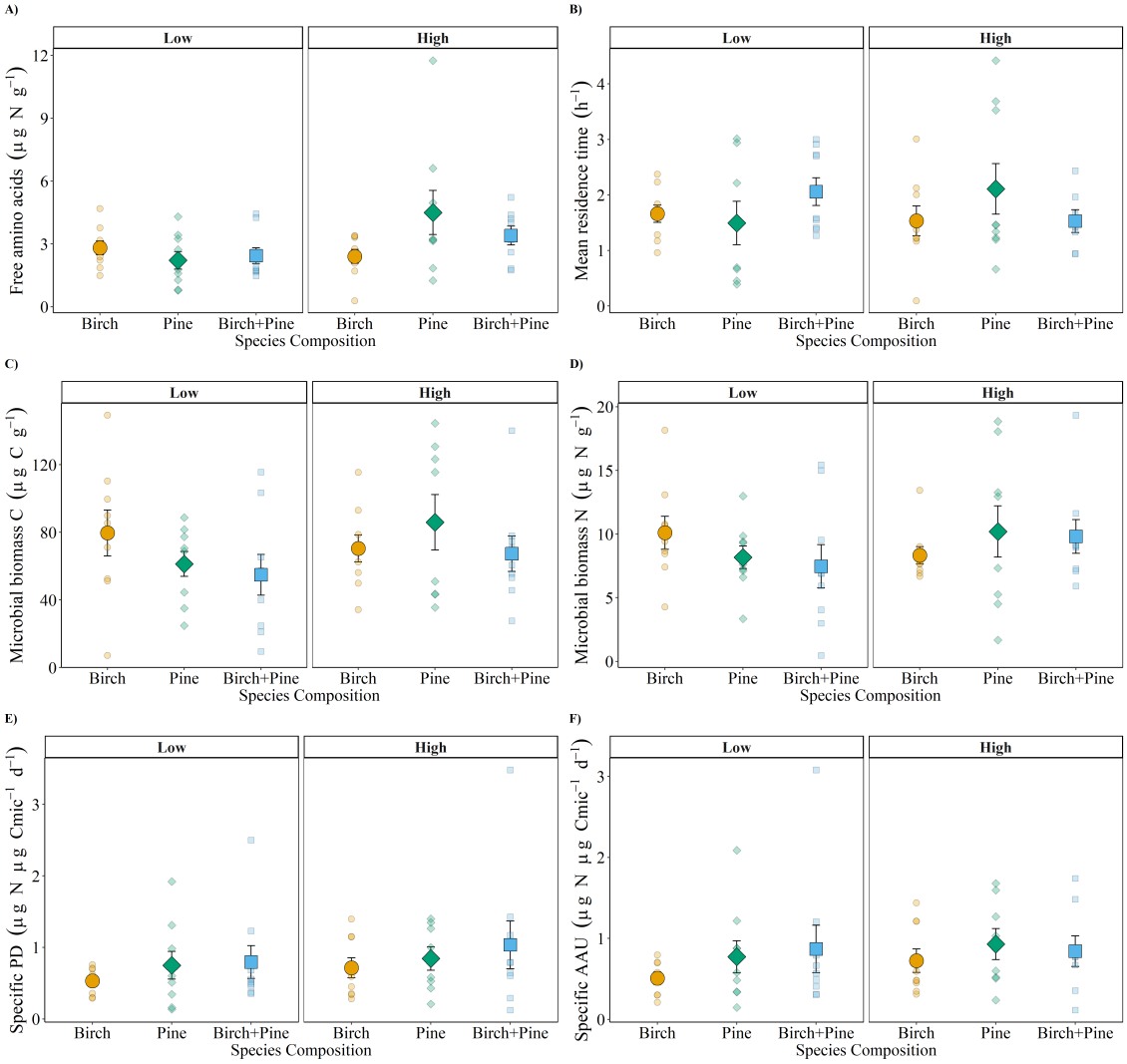

Figure S2.1: Effect of tree species composition and water availability (low *vs.* high) on A) free amino acid concentration and B) mean residence time of free amino acids, C) microbial biomass carbon [C], D) microbial biomass nitrogen [N], E) specific PD activity and F) specific AAU activity. Raw data are presented in partial translucence, with the mean and standard error superposed (n = 9).

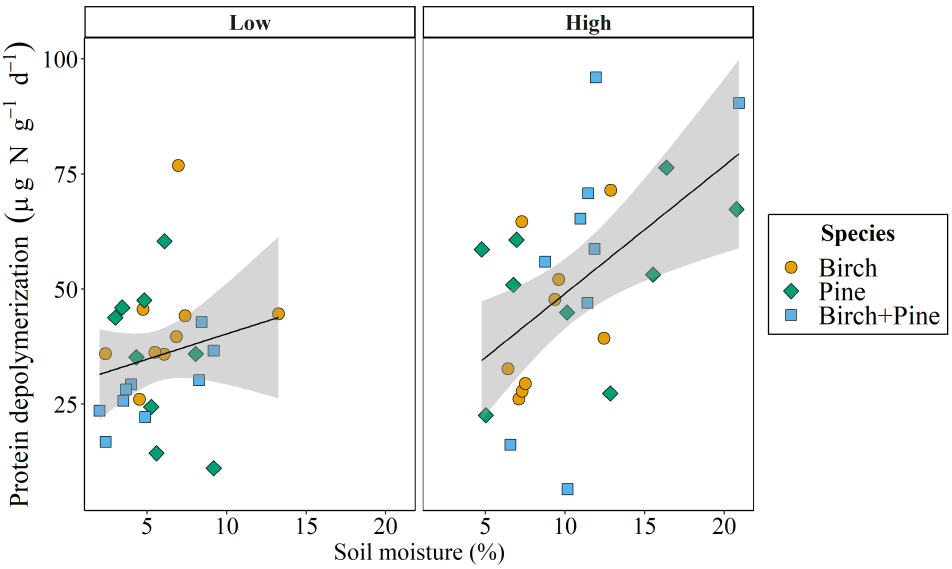

Figure S2.2: Relationship between soil moisture and protein depolymerization for the different species compositions (pure birch, pure pine, mixed birch + pine) in each water availability treatment (low *vs.* high).

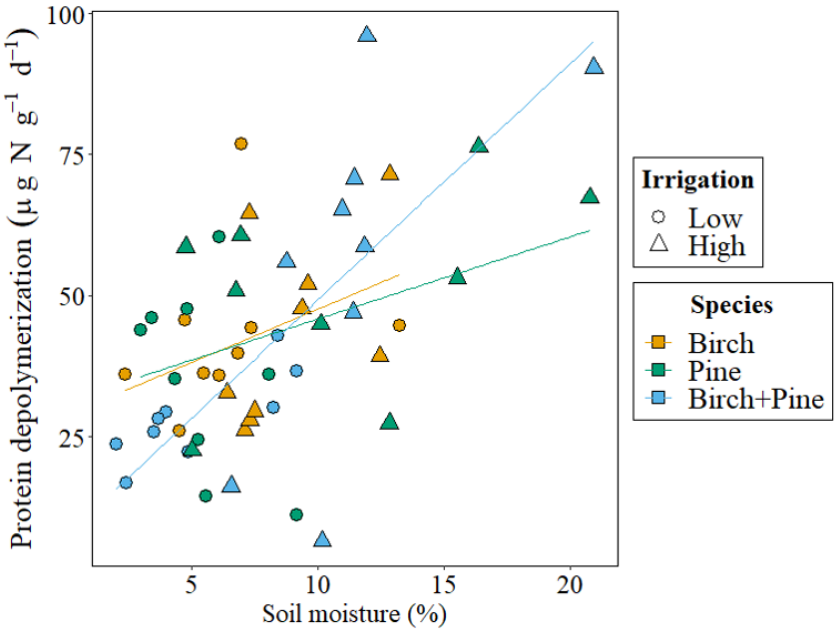

Figure S2.3: Relationship between soil moisture and protein depolymerization for the different species compositions (pure birch, pure pine, mixed birch + pine), including values in both water availability treatments (low *vs.* high).

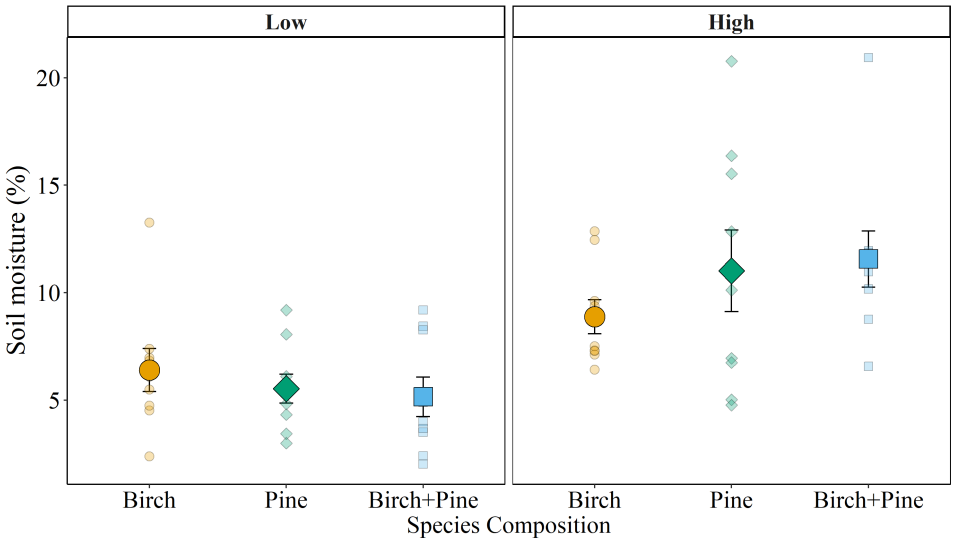

Figure S2.4: Soil moisture (%) per species composition and water availability treatments (low *vs.* high).

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CHAPTER THREE

Tree species composition and nutrient uptake drive soil nutrient availability, even under contrasted levels of water availability

Manuscript soon-to-be submitted by Maxwell, T.L., Fanin, N., Munson, A.D., Lambrot, C., Scolan, L., Trichet, P., & Augusto, L.

Abstract

Temporal asynchrony within a vegetation period, such as when mixing trees with different growth strategies (i.e. deciduous and evergreen trees), may play an important role in accelerating belowground nutrient cycling in mixed-species forests. However, forests are increasingly impacted by drought, and how mixing effects may be modified by changes in water availability is unknown. Using an 11-year-old experimental plantation in south-western France, we tested the interactive effect of stand composition (monocultures of each *Pinus pinaster* and *Betula pendula*, and the mixture of the two species) and water availability (*via* irrigation) on various belowground processes. We quantified soil nitrogen (N) and phosphorus (P) availability with ion-exchange resins in the growing vs dormant periods, net mineralization with an *in situ* soil incubation method every 4-6 weeks from April to September, and N uptake by trees using two ^{15}N soil labeling campaigns, one at budburst and one at peak leaf expansion. The methods were implemented at several time points, in order to elucidate potential temporal complementarity in the mixture, under contrasted levels of water availability. We found that high water availability led to differences in soil nutrient availability between different species compositions, with pure pine having higher mineral N, and pure birch higher available P than the other treatments. These differences were unlikely due to increases in net nutrient mineralization, as these rates were similar among treatments. Instead, differences in tree growth and canopy cover between species compositions, and thus different nutrient uptake needs were the main drivers for the soil nutrient availability trends as indicated by changes in N uptake trends between different stand compositions from budburst to peak vegetation. In the birch-pine mixture, a competition for uptake was demonstrated at budburst, which was then lost once birch's leaves were out in the early summer. We conclude that the timing of nutrient needs and uptake of species within a mixture, such as between a broadleaf deciduous and an evergreen conifer, may be a stronger driver than mineralization for soil nutrient availability in a mixture, even under two contrasted levels of water availability.

Keywords: biodiversity, net mineralization, precipitation, soil nutrient availability, TreeDivNet, uptake

Résumé

L'asynchronie temporelle au cours de la saison de croissance peut jouer un rôle important dans l'accélération du recyclage des nutriments en forêts d'espèces mixtes. Nous avons testé l'effet interactif de la composition du peuplement (bouleau, pin, bouleau-pin) et de la disponibilité en eau (*via* l'irrigation) sur plusieurs processus souterrains. Une forte disponibilité en eau a entraîné des différences dans la disponibilité des nutriments du sol (mesurée à 8 cm) : les pins purs avaient un taux plus élevé d'azote, tandis que les bouleaux purs, plus de phosphore disponible. Cependant, les taux de minéralisation nets étaient similaires parmi les traitements, mais négativement affectés par la forte disponibilité en eau. Enfin, les espèces du mélange présentaient des dynamiques d'absorption différentes, influencées par la croissance et le couvert végétal, mais pas par l'eau. Nous concluons que l'asynchronie de l'absorption des nutriments par les espèces au sein d'un mélange est important pour leur disponibilité.

Mots clés: absorption, biodiversité, disponibilité des nutriments dans le sol, minéralisation nette, précipitations, TreeDivNet

Chapter Introduction

Temporal complementarity

General context

Positive species interactions on soil nutrient availability within diverse forests may be a result of complementarity among the different species (Loreau and Hector 2001), which can be spatial and/or temporal (Forrester 2014). Temporal complementarity can occur with variable rates of nutrient release in mixed compared to monoculture stands throughout the growing season (McTiernan et al. 1997), due to differences in litter qualities and recalcitrance. This complementarity would contribute to a more evenly-timed release of inorganic nutrients throughout the growing season, leading to lower losses and more nutrients available for uptake (McTiernan et al. 1997). Another seasonal mechanism is a difference in timing of activity and nutrient uptake between deciduous and coniferous species, as the activity in the latter can start earlier due to foliage that is functional year-round.

Water availability also plays an important role in controlling belowground processes, as it regulates soil microbial activity (Brockett et al. 2012; Elliott et al. 1981). In forests, limited water supply may reduce litter decomposition rates (Zheng et al. 2017), mineralization (Borken and Matzner 2009), and nitrification (Chen et al. 2011). This decline in process rates yields a decrease in available nutrients (Augusto et al. 2017; Bengtson et al. 2005). However, little is known about how changes in water availability may influence potential temporal complementarity between tree species.

Objective and hypotheses

In this chapter, we explored the potential temporal complementarity of mixing birch and pine on several belowground nutrient processes, under both low and high water availability at the ORPHEE experimental plantations. We tested three main hypotheses (Fig. 3.1):

First, we hypothesized ($H_{3.1}$) that an increase in decomposition with varying leaf litter would lead to an increase in organic matter mineralization (Borken and Matzner 2009) and subsequent higher soil nutrient availability in mixed stands compared to monocultures. More specifically, we expected the mixing effect on soil nutrient availability to be strongest

during the growing period, due to higher microbial activity at this time (Davidson and Janssens 2006).

We further hypothesized ($H_{3.2}$) that asynchrony in nutrient uptake between the evergreen conifer and broadleaf deciduous would be strongest at budburst, due to differences in phenology and growth strategies (Augusto et al. 2014), leading to maximum uptake at this time.

Finally, we hypothesized ($H_{3.3}$) that irrigation would lead to an overall positive effect on process rates, due to its favoring of microbial activity (Brockett et al. 2012). This should be strongest in the peak dry summer, when there would be the largest difference in soil moisture between treatments.

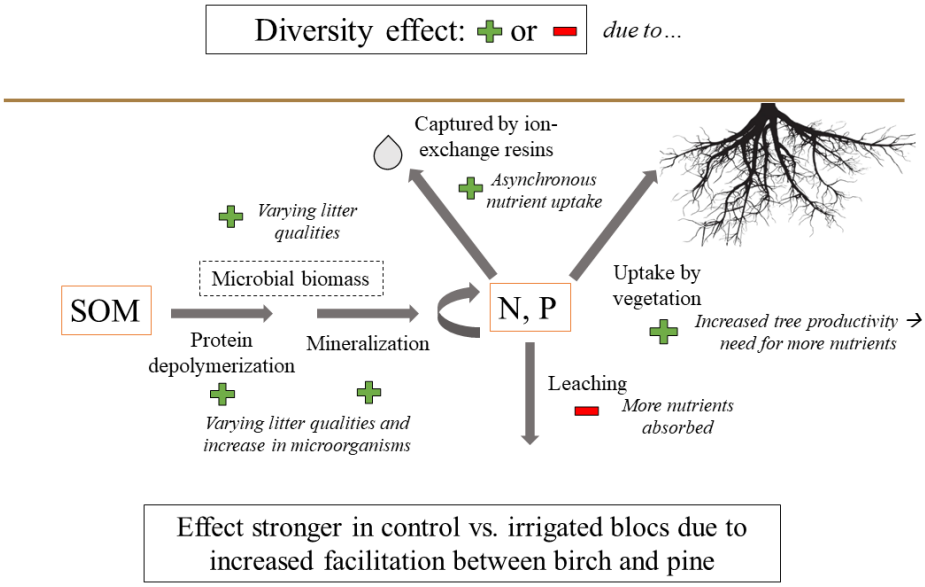

Figure 3.1: Main hypotheses and belowground processes studied in Chapter 3.

Experimental design

The study was based on three-step design (Fig. 3.2): ion-exchange resins were used to estimate soil nutrient availability, Raison's *in situ* soil incubation method for net mineralization rates, and two ^{15}N soil labeling campains (budburst and peak phenology). We studied pure birch, pure pine, and mixed birch-pine under low and high water availability (3 replicated blocks each).

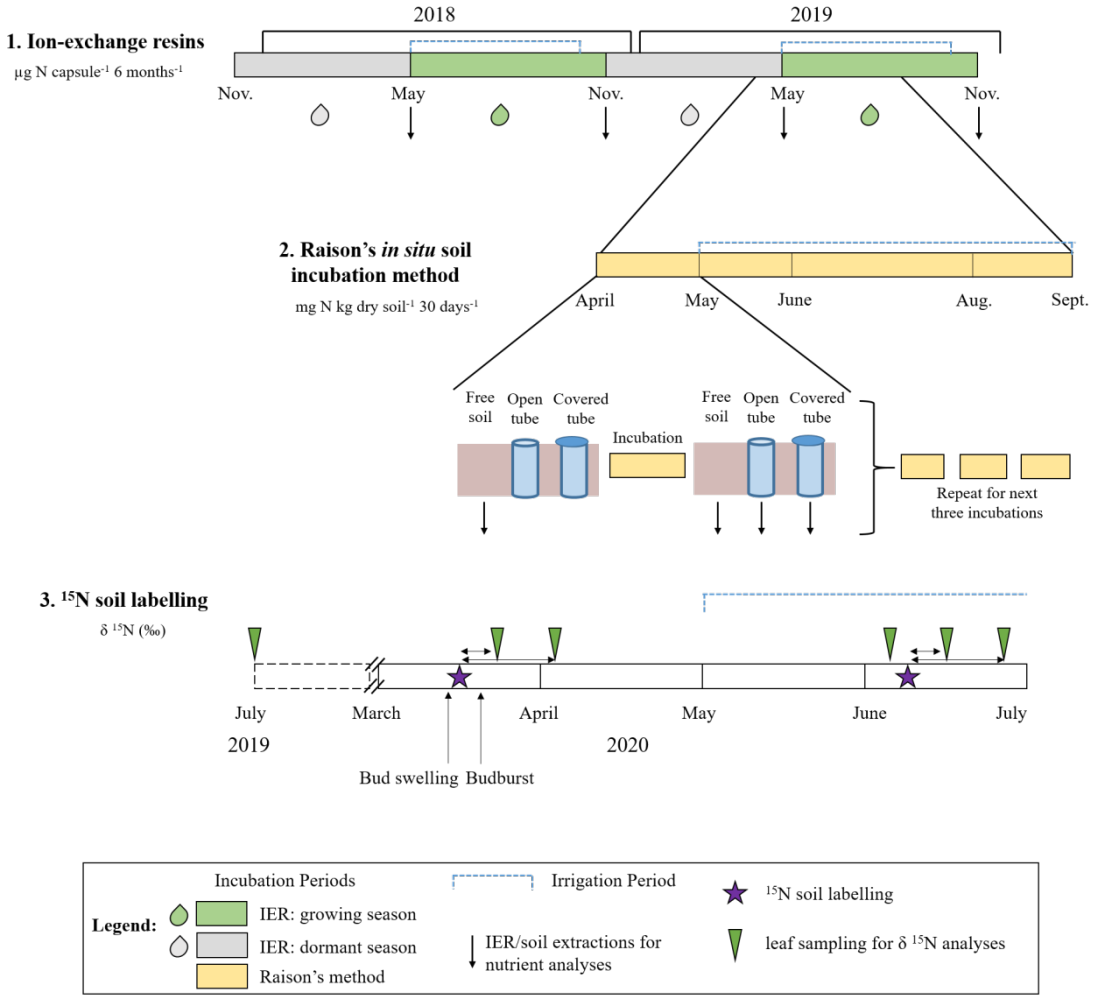

Figure 3.2: Methods overview for Chapter 3.

Main results

Contrary to $H_{3.1}$, we did not find an effect of mixing on soil nutrient availability or net mineralization. However, higher water availability led to differences in soil nutrient availability (measured 8 cm deep) between the different species treatments: pure pine stands were characterized by higher soil mineral nitrogen, while pure birch, higher available phosphorus. Net mineralization rates were similar among species compositions, but negatively impacted by high water availability (contrary to $H_{3.3}$). Finally, species within the mixture had differing uptake dynamics, influenced by growth and canopy cover, but not by water availability (also contrary to $H_{3.3}$). This asynchrony in nutrient uptake was present at budburst, as expected ($H_{3.2}$), but then lost at peak phenology in summer.

Take-home message

We conclude that the timing of nutrient demands and uptake of species within a mixture may be a stronger driver than net mineralization for soil nutrient availability in young mixed forest plantations. Overall, belowground net mineralization and soil nutrient availability were more strongly driven by water availability than by mixing tree species.

3 Paper 3: Tree species composition and nutrient uptake drive soil nutrient availability, even under contrasted levels of water availability

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Soil labeling on June 8th 2020 in a) pure pine, b) pure birch, and c) mixed birch-pine plots. Photos: T. L. Maxwell.

3.1 Introduction

While forests provide a multitude of ecosystem services (Gamfeldt et al. 2013; Jactel et al. 2017; Liang et al. 2016), they are increasingly threatened by more intense and frequent drought periods (IPCC 2014; Schwalm et al. 2012), with important negative consequences on tree productivity and survival (Hartmann et al. 2018). Increasing tree diversity has been a well-explored option to mitigate these negative effects (Lebourgeois et al. 2013; Pretzsch et al. 2013b) while improving ecosystem services such as nutrient cycling (Richards et al. 2010). Indeed, mixed-species forests may be more stable to climatic fluctuations due to species asynchrony and favorable species interactions (Jucker et al. 2014a; Kardol et al. 2018; Morin et al. 2014), which may lead to an increased nutrient supply (Richards et al. 2010) and associated productivity as compared to pure stands (Baeten et al. 2019; Cardinale et al. 2007; Liang et al. 2016; Zhang et al. 2012). It is thus of particular interest to investigate how changes in water availability may influence the effects of tree diversity on nutrient availability. While biodiversity-ecosystem functioning relationships have been found to be stronger in drier climates (Ratcliffe et al. 2017), we know little about how limiting water conditions may affect biodiversity benefits and how their interactive effects may affect belowground nutrient cycling.

Positive species interactions on soil nutrient availability within diverse forests may be a result of complementarity among the different species (Loreau and Hector 2001), which can be spatial and/or temporal (Forrester 2014). Spatial complementarity in forests may occur due to niche partitioning along the soil profile (Brassard et al. 2013) or depth-specific asymmetric root competition (Altinalmazis-Kondylis et al. 2020). A commonly studied trait of mixed species forests is the temporal stability of production over time, at both the community and individual tree levels (del Río et al. 2017; Forrester 2014; Morin et al. 2014). This is often explained due to the asynchrony in canopy dynamics (Martin-Blangy et al. submitted), favoring light interception by the different tree species (Jucker et al. 2014b; Pretzsch et al. 2014). When discussing temporal complementarity on belowground nutrient cycling, the asynchrony within growing seasons is relatively unknown, yet this smaller scale data can inform us of the belowground functioning in mixed species forests.

Mixing trees with different growth strategies (i.e. pioneer species *vs.* late-successional species), nutrient needs, and timing for the exploitation of these nutrients, may lead to seasonal complementarity of belowground N cycling (Rothe and Binkley 2001). In general, net N mineralization is expected to be higher in deciduous forests than in coniferous forests (Côté et al. 2000; Gosz 1981; Melillo 1981), due to the nutrient-rich litter stimulating belowground microbial activity, although other factors such as the indirect effect on soil properties or understory communities can also play a role affecting mineralization (Augusto et al. 2015; Brierley et al. 2001; Lejon et al. 2005). Mixing broad-leaved with

needle litters is the mixture that has most often been found to increase N mineralization beyond predicted values (Chapman et al. 1988; Fyles and Fyles 2011; Gartner and Cardon 2004; McTiernan et al. 1997; Salamanca et al. 1998; Williams and Alexander 1991), as a species with nutrient-rich litter may enhance the decomposition and nutrient release from species with low-quality litter (Chapman et al. 1988; Finzi and Canham 1998; Wardle et al. 1997). More recently, studies have highlighted the importance of functional diversity for litter decomposition (Mao et al. 2017), and that both synergistic and antagonist interactions between mixed-species litter may decrease in magnitude with increasing similarity between the litter and present tree species (Jewell et al. 2015). Additionally, Liu et al. 2020 found that mixing effects on litter decomposition were influenced by nutrient transfer, soil fauna, and inhibitory secondary compounds, and that the overall mixing effect is an interplay between concurrent synergistic and antagonistic effects.

Temporal complementarity can occur with variable rates of nutrient release in mixed compared to monoculture stands throughout the growing season (McTiernan et al. 1997), due to above-mentioned differences in litter qualities and recalcitrance. This complementarity would contribute to a more evenly-timed release of inorganic nutrients throughout the growing season, leading to lower losses and more nutrients available for uptake (McTiernan et al. 1997). Another seasonal mechanism is a difference in timing of activity and nutrient uptake between deciduous and coniferous species, as the activity in the latter can start earlier due to foliage that is functional year-round. A change in nutrient phenology of the species in the mixture compared to those in the monoculture could also lead to differences in timing of nutrient uptake, generally reducing resource losses from the ecosystem (Knops et al. 2002). The question of whether and how mixing tree species, such as by seasonal temporal complementarity, may specifically increase nutrient resources captured, has not been yet been answered (Forrester 2014). Studies have focused on root biomass and nutrient concentrations (Lang and Polle 2011) or aboveground nutrient content (Oelmann et al. 2010) as estimations for nutrients captured in mixed species forests. Even less is known about how these temporal mixing effects may be impacted by changes in water availability.

Water availability also plays an important role in controlling belowground processes, as it regulates soil microbial activity (Brockett et al. 2012; Elliott et al. 1981). Prolonged droughts contribute to decreased soil moisture, which subsequently can cause a reduction in soil microorganism activity in aerobic conditions (Kreuzwieser and Gessler 2010). In forests, limited water supply may reduce litter decomposition rates (Zheng et al. 2017), mineralization (Borken and Matzner 2009), and nitrification (Chen et al. 2011). This decline in process rates yields a decrease in available nutrients (Augusto et al. 2017; Bengtson et al. 2005). The negative effects of drought on microbial processes can be compensated by an increase in rates following rainfall (He and Dijkstra 2014). While

drier soils can present slower microbial activity, the effect of increasing soil moisture may be constrained by soil nutrient availability (Cusack et al. 2019). Additionally, lower precipitation can exacerbate the effect of N inputs, which can lead to greater plant N uptake, soil N availability and forest ecosystem N losses (Rivero-Villar et al. 2021). However, little is known about how limiting water conditions may affect the expected beneficial effect of mixing tree species, especially on belowground nutrient cycling.

We tested the interactive effect of mixing tree species and water availability on various belowground nutrient cycling processes, in order to elucidate potential seasonal complementarity at varying temporal resolutions. To do so, the study was conducted on a unique experimental design in southwestern France (ORPHEE) because it manipulates *in situ* both the mixing of functionally different tree species (in this study, birch and pine) and water availability (*via* irrigation). We hypothesized (H_1) that asynchrony between species would lead to more rapid nutrient cycling, with an increase in organic matter mineralization (Borken and Matzner 2009) and subsequent higher soil nutrient availability in mixed stands compared to monocultures. More specifically, we expected the mixing effect on soil nutrient availability to be strongest during the growing period, due to higher microbial activity at this time (Davidson et al. 2006). We further hypothesized (H_2) that asynchrony in nutrient uptake between the evergreen conifer and broadleaf deciduous would be strongest at budburst, due to differences in phenology and growth strategies (Augusto et al. 2014), leading to maximum uptake at this time. Finally, we hypothesized (H_3) that irrigation would lead to an overall positive effect on process rates, due to its favoring of microbial activity (Brockett et al. 2012). This should be strongest in the peak dry summer, when there would be the largest difference in soil moisture between treatments.

3.2 Materials and methods

3.2.1 Site description

The ORPHEE experiment is located in southwestern France (44°44.35’ N, 00°47.9’ W, elevation of 60 m asl.; Castagneyrol et al. 2013). The mean annual temperature is 12.5°C and the mean average precipitation is 870 mm (1993 - 2018), but most precipitation occurs from the fall to the spring, often leading to dry summers considered representative of low water availability in this region. Plantation occurred in 2008, after a clear-cut of a *Pinus pinaster* forest with a single plough and fertilizer application of 60 kg of P_2O_5 and 60 kg of K_2O per ha. The predominant soil type is a podzol characterized by a coarse texture (95% sand) and low soil fertility (Augusto et al. 2010), particularly in phosphorus (Trichet et al. 2009). The very coarse texture of the soil negatively affects its water holding capacity

with values that is typically only ~50 mm down to 1 meter (Augusto et al. 2006; Jolivet et al. 2007). A total of 256 plots were planted, with five species (*Betula pendula* Roth., *Pinus pinaster* Soland. in Aït., *Quercus ilex* L., *Quercus pyrenaica* Willd., *Quercus robur* L.) in all possible combinations from one (monocultures) to five species mixtures. We chose to work with three species composition treatments: pure silver birch (*Betula pendula* [Be] Roth.) plots, pure maritime pine (*Pinus pinaster* [Pp] Soland. in Aït.) plots, and two-species mixed birch-pine plots (used to study the effect of tree mixing). Pine and birch were selected because, contrary to the other tree species, they grew more successfully under the harsh conditions imposed by understory competition, dry summers, and the low fertility of the sandy soil, resulting in relatively dense stands. Each plot has 100 trees within a 20 m by 20 m area. Trees were arranged in a substitutive (same tree density irrespective of the composition) alternate (trees from one species have neighbors from all other associated species) pattern. The plots are separated by 3 m from one another, and randomly located within six blocks: three control (dry) blocks and three irrigated blocks. The irrigation was started in 2015, and occurs seasonally from early May to late October/early November to alleviate summer water stress. The water is taken directly from the groundwater, which has low concentrations of nitrogen and phosphorus, but higher concentrations of other elements, such as calcium and magnesium (Jolivet et al. 2007). A two-meter-tall sprinkler installed in the center of each experimental plot sprays 3 mm per night. On average, control blocks received 318 mm of water from early May to late September, while irrigated blocks received 777 mm of water (rain + irrigation) during the same period. When using both temperature and precipitation (+ irrigation) to determine arid periods in an ombrothermic diagram (Walter and Lieth 1967), control blocks are subjected to more stress than irrigated blocks (Fig. S3.1).

3.2.2 Stand biomass measurements

Aboveground biomass of trees, and the understory, were estimated in 2018. The tree diameter at 1.3 m aboveground for all living trees within the central core area of the plot (i.e. excluding two perimeter rows of trees), and allometric equations for pine (Shaiek et al. 2011) and birch (Johansson 1999), were used to calculate an average biomass for each species in their monoculture and mixture. The understory biomass was measured by a phytovolume method (Gonzalez et al. 2013; Vidal et al. 2019). Briefly, each plot was divided into four zones, and the following present understory species were quantified: *Pteridium aquilinum L. Kuhn, Molinia caerulea L. (Moench), Calluna vulgaris L. Hull, Erica cinerea L., Erica scoparia L., Frangula alnus P. Mill., Ulex europaeus L., Ulex minor Roth., Rubus fruticosus L.,* and *Lonicera periclymenum L.* Their aboveground cover percentage was estimated, and average height and maximum height were measured (Vidal et al. 2019). We then calculated a sum of all understory aboveground biomass for each

zone, and calculated an average per plot from the four zones.

3.2.3 Experimental overview

The study of the nutrient availability and uptake was based on a three-approaches design (see Fig. 3.2 for a detailed description). We placed (1) ion-exchange resins (IER; PST-1 Capsule, UNIBEST), which captured the long-term nutrient accumulation over four periods of 6 months (hereafter referred to as "growing season" and "dormant season", with May and November as start/end months), from November 2017 to November 2019 (Fig. 3.2). In order to better understand the mechanisms behind nutrient accumulation in the resins during the vegetation period, we also installed (2) *in situ* soil incubations following Raison's method (Raison et al. 1987). We were able to estimate net mineralization, root uptake and leaching rates during these shorter incubation periods from April to September 2019 (Fig. 3.2). Finally, we applied (3) a ^{15}N labelled solution to the soil in order to estimate nutrient uptake at budburst and at peak phenology in early summer in 2020 (Fig. 3.2). All of the methods are described below.

3.2.3.1 Ion-exchange resins

Ion exchange resins (IER; PST-1 Capsule, UNIBEST), were installed to estimate accumulated NH_4^+, NO_3^- and PO_4^{3-} in soils. In each plot, four IER were placed in the top soil (at 8 cm deep, the 0 cm level being the limit between the top of the mineral layers and the bottom of the forest floor) on November 11th 2017, and left for six months in the field with a metal grid to prevent wild boars from disturbing the soil, allowing the accumulation of available nutrients with a sensitivity to on-site factors (Binkley and Matson 1983). The extraction of the resins allowed for the quantification of accumulated available N and P during two periods of the year, in the dormant season ($\approx$ Oct./Nov. – April/May period) and the growing season ($\approx$ April/May – Oct./Nov. period). On April 3rd 2018, they were collected and new resins placed nearby. The IER were extracted by 1 M KCl and analyzed by colorimetric determination (SKALAR, San ++). This was repeated three times (analysis and installment of new resins on November 21st 2018, and May 15th 2019, finishing on October 16th 2019). Results were standardized to correspond to µg capsule^{-1} 6 months^{-1} for each incubation period. Mineral N is hereto referred to as the sum of $N-NH_4^+$ and $N-NO_3^-$.

3.2.3.2 Raison's *in situ* soil incubation method

To study soil process rates, we installed *in situ* incubation tubes to estimate net mineralization rates in all of the studied plots (Raison et al. 1987). This well-established

method is able to detect small short-term (monthly to seasonal) change and cumulative effects of varying managements on soil mineral N dynamics (Khanna and Raison 2013). The monitoring was carried out in spring-summer 2019, which is a period representative enough to capture the whole year dynamics (Schaffers 2000).

Briefly, a set of undisturbed soil in tubes (depth: 18cm, diameter: 8cm) was installed in the soil and kept uncovered and another set was kept covered with a lid during field incubation. The mineral nutrient contents were measured in the unconfined soil at the time of installation of the incubation tubes. After around 5 - 8 weeks (exact dates detailed below), the mineral nutrient contents in both covered and uncovered tubes were measured by water extraction and colorimetric analysis (SKALAR, San ++). The net rates for each nutrient [Nut] were calculated as below, with T2 corresponding to soil after the incubation and T1 to soils measured prior to incubation.

$$Net\ nut.\ mineralization = covered\ tube\ [Nut]_{T2} - unconfined\ soil\ [Nut]_{T1} \tag{3.1}$$

$$Net\ nut.\ leaching = covered\ tube\ [Nut]_{T2} - uncovered\ tube\ [Nut]_{T2} \tag{3.2}$$

$$Net\ nut.\ uptake = uncovered\ tube\ [Nut]_{T2} - unconfined\ soil\ [Nut]_{T2} \tag{3.3}$$

At the harvest date, the tubes were reinstalled nearby but in an undisturbed area, and the net mineralization, uptake and leaching were calculated again after another six weeks (incubation times differed, but rates were standardized to 30 days^{-1} in order to be comparable between each other). This was repeated for a total of four times, in order to have estimations of net mineralization rates at four periods from early spring to late summer, corresponding to different soil conditions: fresh and moist soils with moderate microbial activity (April 1st – May 9th), warm and still humid soils with strong activity (May 9th – June 17th), dry soils with low activity (June 17th – August 21st), and even drier soils during the fourth period (August 21st – September 26th). Three sets of tubes were set up in each plot, and the soils from these three field replicates were homogenized in the field to produce one sample for each of the free soil, covered tubes and uncovered tubes per plot at each date. Samples were stored at 4 °C until analysis in the subsequent days.

Fresh soils were sieved to 2 mm, homogenized, and 1 aliquot per tube type per plot was extracted at each date (3 tube types x 3 species compositions x 2 water treatments x 3 blocks per water treatment = 54 samples per date). Briefly, 50 mL of ultrapure water was added to a glass vial with the 10 g aliquot, which was then shaken for 16 h at 4 °C. Water extraction was favored over KCl extraction due to potential interference of the

extractant with available phosphate in sandy podzols (Achat 2009), and because it has a similar extraction efficiency for mineral N as in saline solutions (Gallet-Budynek, INRAE; personal communication). The nutrients (NH_4^+, NO_3^- and PO_4^{3-}) were analyzed in the extractant by colorimetric determination (SKALAR, San ++). Rates were calculated for each nutrient, and then the sum of the $N-NH_4^+$ and $N-NO_3^-$ were summed to produce one overall 'net mineral N' value. Due to the high intra-plot variability and high number of uptake and leaching rates that were calculated to be negative and thus converted to zero, we did not proceed to analyzing these two variables (presented in Fig. S3.3 and Fig. S3.4).

3.2.3.3 ^{15}N soil labeling

Two pairs of trees were selected per plot, one pair for the budburst labeling and the other for the summer labeling. The surrounding aboveground understory was cleared in an area of 3.5 m × 1.5 m around the pair of trees, which were 2 m away from one another. After the soil was bare, a wooden quadrant and plastic sticks were used to accurately measure the area to be labeled (3 m × 1 m) around each pair of trees per plot. The labeling solution was prepared with 55 mg of $^{15}NH_4Cl$ in 20 mL of deionized water in the laboratory. At the field, this was further diluted to 2 L of deionized water. The solution was sprayed over the labeling surface (3 L of 0.075 M, a non-fertilizing dose, over 3 m^2), so that the soil was labeled with 7.5 mg ^{15}N per m^2. The 3 L were sprayed evenly, by spraying 250 mL at a time, in each of the 12 measured squares of 50 cm × 50 cm using a small watering can. Labeling occurred on March 16th around budburst, and on June 8th 2020, on the second pair of trees per plot, located at least 5 m away from the first pair labeled in March.

In August 2019, green leaves from several inner trees were sampled in order to get a baseline $\delta^{15}N$ value for each species in each plot prior to the labeling experiment happening right around budburst. Green leaves for each of the two trees in the labelling area were collected at two locations in the upper canopy using an extendable pruning shear. This was done 1 week and 3 weeks after labeling, to observe any additional dynamics. For the second labeling date, green leaves were sampled a few days before the labeling occurred, to account for potential contamination from the previous nearby labeling experiment. The green leaves were also sampled 1 and 3 weeks after labeling. Leaves for each tree at each sampling date were placed in individual paper bags and dried in an oven at 40°C for at least 72 hrs. One composite was made from the two trees of each monoculture, so that per block there was 1 birch sample from its monoculture, 1 pine sample from its monoculture, 1 birch sample from the mixture, and 1 pine sample from the mixture. Each composite was cut up and ground, until the sample became a homogeneous powder. All material was wiped down with ethanol between samples to avoid contamination. A 2 mg aliquot of the powder was then weighed into tin caps on a microgram balance. The samples were analyzed for their $\delta^{15}N$ using an Elemental Analyzer – Isotopic Ratio Mass Spectrometer

(EA-IRMS), in the SILVATECH platform (Structural and functional analysis of tree and wood Facility) from the research center INRAE Grand-Est Nancy.

3.2.4 Statistical analyses

All of the statistical analyses were done using R software (version 4.1.0). First, extreme values were removed following the outlier labelling rule with a conservative tuning parameter of g = 2.2 (Hoaglin and Iglewicz 1987). On average, less than 5% of the IER values were removed, and no values were removed from the aboveground biomass, Raison's method, nor the $\delta^{15}N$ data.

Mixed models were used to assess the effect of the following fixed effect factors: tree species composition (pure birch, pure pine, mixed birch-pine), water availability (irrigation, control), and their interaction, on the available nutrients extracted from the IER, mineralization rates, and understory biomass. Pairs of control and irrigated blocks (i.e. one 'Superblock') were included as a random effect to account for the spatial structure of our experimental design, with water availability treatment and Plot nested into nested into 'Superblock' (for IER only, as there were replicates per plot). The corAR1 autocorrelation function was used to take into account correlation between the four time points, with date as the random slope and the spatial random effects as the random intercepts. For the $\delta^{15}N$ and tree aboveground biomass data, we had values per species within the mixture, so a mixed-effect model was used to test the overall effect of mixing (monoculture, mixture), species (birch, pine), irrigation (irrigation, control), and their interaction on the $\delta^{15}N$ values or tree aboveground biomass data. The $\delta^{15}N$ data set was separated into the two labeling dates, as individuals differed between the budburst and summer labeling experiments. Similar to the previous model, the model included a spatial random effect (plot nested in water availability treatment, nested in Superblock) and corAR1 autocorrelation function was to take into account correlation between the three sampling dates (Pre-label, post 1-week and post 3-week). Only the spatial random effect was implemented in the model for tree aboveground biomass, as only one date was analyzed.

All mixed-effect models were coded using the *'nlme'* package (Pinheiro and Bates 2020, October 24) with $\alpha = 0.05$ as significance level. Variables were log-transformed if needed to improve the normality of residuals. For all three data types and model results, we then applied contrasts and performed Tukey's HSD tests using the *'multcomp'* package (Hothorn et al. 2008), with $\alpha = 0.05$ used as the level to define significance. Data was organized using the *'dplyr'* package (Wickham et al. 2021) and graphs were produced with the *'ggplot2'* package (Wickham 2016).

Table 3.1: Results of the mixed model analyses of species composition (Sp), water availability treatment (Irr) and their interaction on understory aboveground biomass, soil nutrient availability and net mineralization rates. For the tree aboveground biomass and $\delta^{15}N$ uptake values, results come from the mixed model analysis of diversity (Div), Sp, Irr, and their interactions. Numerator and denominator degrees of freedom, as well as F-values and p-values for fixed effects are presented. Significant ($p < 0.05$) effects are noted in bold font.

Variable	Fixed effect	numDF	denDF	F-value	p-value
Tree aboveground biomass	Div	1	21	77.90	**< 0.001**
	Sp	1	21	583.93	**< 0.001**
	Irr	1	21	10.49	**0.004**
	Div x Sp	1	21	0.38	0.546
	Div x Irr	1	21	4.49	**0.046**
	Sp x Irr	1	21	0.00	0.994
	Div x Sp x Irr	1	21	0.16	0.696
Understory aboveground biomass	Sp	2	15	23.61	**< 0.001**
	Irr	1	15	0.15	0.702
	Sp x Irr	2	15	0.03	0.973
Mineral N ($N\text{-}NH_4^+ + N\text{-}NO_3^-$)	Sp	2	10	4.46	**0.041**
	Irr	1	10	41.43	**< 0.001**
	Sp x Irr	2	10	2.23	0.158
Mineral P ($P\text{-}PO_4^{3-}$)	Sp	2	10	1.93	0.195
	Irr	1	10	9.01	**0.013**
	Sp x Irr	2	10	3.03	0.093
Net N mineralization	Sp	2	10	0.22	0.803
	Irr	1	10	8.29	**0.016**
	Sp x Irr	2	10	0.29	0.754
Net P mineralization	Sp	2	10	0.28	0.762
	Irr	1	10	9.17	**0.013**
	Sp x Irr	2	10	0.27	0.768
Budburst ^{15}N uptake	Div	1	14	1.17	0.298
	Sp	1	14	82.24	**< 0.001**
	Irr	1	14	0.95	0.346
	Div x Sp	1	14	3.34	0.089
	Div x Irr	1	14	0.00	0.974
	Sp x Irr	1	14	0.21	0.655
	Div x Sp x Irr	1	14	0.47	0.502
Summer ^{15}N uptake	Div	1	14	0.60	0.450
	Sp	1	14	26.68	**< 0.001**
	Irr	1	14	3.75	0.073
	Div x Sp	1	14	0.62	0.443
	Div x Irr	1	14	0.00	0.964
	Sp x Irr	1	14	0.00	0.972
	Div x Sp x Irr	1	14	0.05	0.818

3.3 Results

3.3.1 Aboveground biomass

In general, pine had significantly higher tree aboveground biomass than birch ($p < 0.001$, Table 3.1, Table 3.2). Mixing birch and pine led to an overall positive non-additive effect on biomass in both control and irrigated plots ($p < 0.001$, Table 3.2). While the theoretical additive value of mixing in control plots was 5.84 ± 1.26 kg m^{-2} (mean ± s.e.), the observed tree aboveground biomass was significantly higher, at 8.23 ± 0.75 kg m^{-2} (Table 3.2). Under irrigation, the difference was smaller from the theoretical additive effect (7.22 ± 1.2 kg m^{-2}) to the observed effect (8.81 ± 0.11 kg m^{-2}, Table 3.2). Irrigation had an overall positive effect on tree aboveground biomass ($p = 0.004$, Table 3.1).

The understory aboveground biomass at our experimental site significantly differed between species composition treatments ($p < 0.001$, Table 3.1, Table 3.3). Pure birch had three times more understory biomass than pure pine, in both control and irrigated plots (Table 3.3). The understory accounted for around 2% of total aboveground biomass (sum of understory and tree aboveground biomass) in pure birch plots, less than 0.25% in pure pine, and 0.35% in the mixed birch-pine plot.

Table 3.2: Tree aboveground biomass for birch (*Betula pendula*, Be) and pine (*Pinus pinaster*, Pp) grown in their monoculture and in the two-species mixture, under both control and irrigation water availability treatments. Values are mean biomass per tree per m^2 (± standard error), with measures taken in 2018. For each irrigation treatment, the total biomass per tree per m^2 (sum of birch and pine biomass) is presented in bold font.

Irrigation	Target	Diversity	Species	n	Tree aboveground biomass (kg m^{-2})
Control	Be	Monoculture	Be	4	2.6 ± 0.33
	Pp	Monoculture	Pp	4	9.07 ± 0.52
	Be	Mixture	Be-Pp	4	1.14 ± 0.16
	Pp	Mixture	Be-Pp	4	7.09 ± 0.66
	Total	**Mixture**	**Be-Pp**	**4**	**8.23 ± 0.75**
Irrigated	Be	Monoculture	Be	4	4.08 ± 0.28
	Pp	Monoculture	Pp	4	10.35 ± 0.35
	Be	Mixture	Be-Pp	4	1.33 ± 0.3
	Pp	Mixture	Be-Pp	4	7.48 ± 0.22
	Total	**Mixture**	**Be-Pp**	**4**	**8.81 ± 0.11**

Table 3.3: Understory aboveground biomass in each of the treatments (mean ± standard error), estimated in kg per m^2 for measures taken in 2018.

Irrigation	Diversity	Species	n	Understory aboveground biomass (kg m^{-2})
Control	Monoculture	Be	4	0.068 ± 0.010
Control	Monoculture	Pp	4	0.026 ± 0.009
Control	Mixture	Be-Pp	4	0.030 ± 0.004
Irrigated	Monoculture	Be	4	0.068 ± 0.007
Irrigated	Monoculture	Pp	4	0.023 ± 0.006
Irrigated	Mixture	Be-Pp	4	0.027 ± 0.004

3.3.2 Soil nutrient availability

Overall, mineral N extracted from the ion exchange resins (IER) was affected by both species composition ($p = 0.041$) and by irrigation ($p < 0.001$; Table 3.1, Fig. 3.3A). When sub-setting per water availability treatment, differences between species compositions emerged only under irrigation, where pine had significantly higher mineral N than both pure birch ($p = 0.005$) and the birch-pine mixture ($p = 0.009$; Table S3.1, Fig. 3.3A). For all incubation periods combined, pine had on average 168% more N recovered in the IER than pure birch, and 197% more than in the mixture in irrigated plots (Fig. 3.3A). When considering all species compositions together, N in irrigated plots was reduced by 63% compared to control plots (Fig. 3.3A).

Mineral P extracted from IER was negatively affected by irrigation ($p = 0.013$), on average by 46% (Table 3.1, Fig. 3.3B). When sub-setting for each water availability treatment, birch had significantly higher P than both pine ($p = 0.038$) and the birch-pine mixture ($p = 0.002$) under irrigation (Table S3.1, Fig. 3.3B). Overall, this corresponded to 84% more P extracted under birch than in pure pine, and 123% more than in the mixture in the irrigated plots.

Under control plots, no significant differences between species composition treatments were observed (Table S3.1, Fig. 3.3B). For both N and P, models including season as a fixed factor revealed a significant effect of this effect ($p < 0.001$), with more nutrients recovered during the growing season than during the dormant season (Fig. 3.3).

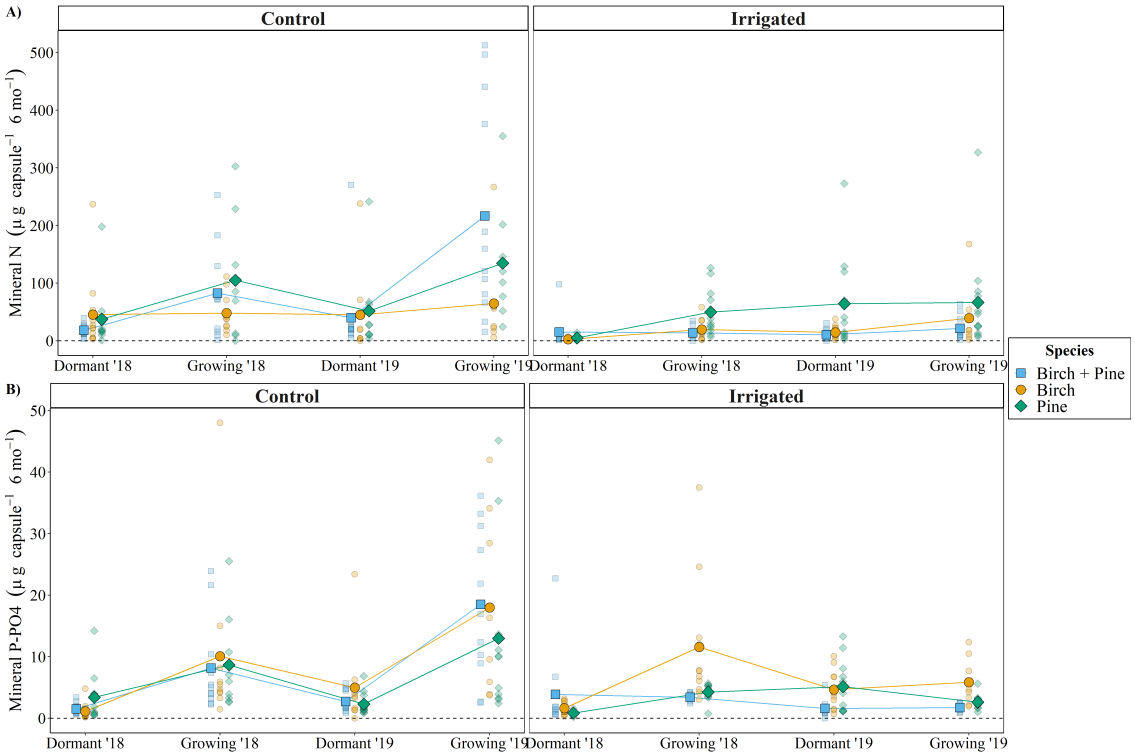

Figure 3.3: Soil mineral N ($N-NH_4^+$ + $N-NO_3^-$) and $P-PO_4^{3-}$ concentrations in mixed birch + pine (squares, *blue*), pure birch (circles, *yellow*), and pure pine plots (diamonds, *green*) under both control and irrigated water availability treatments. At each incubation period (dormant and growing seasons of 2018 and 2019), the raw data are presented in partial translucence, with the mean value superposed (n = 12).

3.3.3 Net nutrient mineralization

No significant effects of species composition, or its interaction with water availability, were found when analyzing the net nutrient mineralization rates estimated with Raison's *in situ* incubation method (Table 3.1, Fig. 3.4). Net nutrient mineralization values above 0 indicated net mineralization and negative values indicated net immobilization by soil microorganisms (Fig. 3.4). Overall, there was a slight negative effect of irrigation on net N and P mineralization, i.e. nutrients were generally more immobilized than mineralized in irrigated compared to control plots (p = 0.013 for N and p = 0.016 for P, Fig. 3.4). The patterns between the net mineralization of N and P were almost identical, with net N mineralization/immobilization being on average around three times greater than net P mineralization/immobilization (Fig. 3.4). On average, N mineralization rates ranged from 0.64 ± 0.24 mg N kg^{-1} month^{-1} (mean ± s.e.) in control plots to -0.26 ± 0.23 mg N kg^{-1} month^{-1} in irrigated plots (Fig. 3.4A). Phosphorus mineralization rates ranged from 0.20 ± 0.07 mg P kg^{-1} month^{-1} in control plots to -0.09 ± 0.07 mg P kg^{-1} month^{-1} in irrigated plots (Fig. 3.4B).

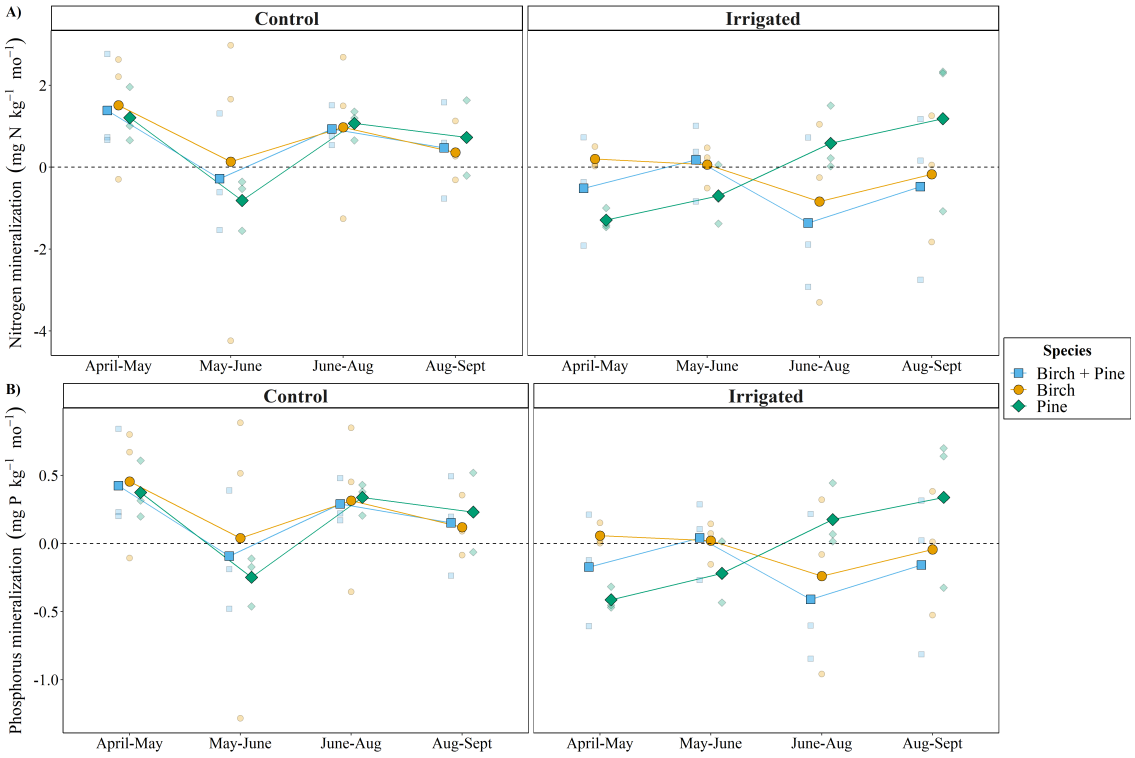

Figure 3.4: Mineral N and P mineralization in mixed birch + pine (squares, *blue*), pure birch (circles, *yellow*), and pure pine plots (diamonds, *green*) under both control and irrigated water availability treatments. Nutrient mineralization values above 0 indicate net mineralization, and values below 0 indicate immobilization. At each incubation period in 2019, the raw data are presented in partial translucence, with the mean value superposed (n = 3).

3.3.4 Uptake

At both the budburst and summer 2020 labeling experiments, pine needles had significantly higher $\delta^{15}N$ values than birch leaves ($p < 0.001$ for both seasons, Table 3.1, Fig. 3.5). This was also true for the leaves from the pre-labeling leaf sampling analysis in summer 2019, for which birch had a $\delta^{15}N$ value of -0.87 ± 0.28 (mean ± s.e.) and pine had higher values, around 6.82 ± 0.65 $\delta^{15}N$ (Fig. 3.5 'Pre-label' in budburst panels).

After the budburst labeling, pine in its monoculture had lower values than prior to labelling, dropping to 2.98 ± 0.48 $\delta^{15}N$ one week post-labeling and subsequently staying stable with values at 3.26 ± 0.33 $\delta^{15}N$ at the post-three-week sampling (Fig. 3.5). Irrigation did not affect pine's uptake at this time in the season. In the monoculture, birch at budburst had a higher $\delta^{15}N$ three weeks after labeling than prior, with values slightly higher in control plots, at 2.09 ± 1.55 $\delta^{15}N$, than in the irrigated plots, at -0.13 ± 0.27 $\delta^{15}N$ (Fig. 3.5). Notably, the overall model for budburst showed a trend for an interactive effect between mixing and species ($p = 0.089$): mixing increased pine $\delta^{15}N$ while it decreased birch $\delta^{15}N$ compared to each species in their respective monocultures (Table 3.1). This was especially true in control plots, where mixing increased pine $\delta^{15}N$ by 48% while decreasing birch $\delta^{15}N$ by 121%. However, no overall effect of irrigation at budburst came out of the general

model (Table 3.1).

During the summer labeling experiment, the effects of mixing were not observed; both species behaved similarly in their monoculture and in the mixture (Fig. 3.5). Both species also showed a strong increase in $\delta^{15}N$ one-week after labeling, a signal that stayed constant at the post-three-week sampling. On average, pine $\delta^{15}N$ increased by 264% one week after labeling, while birch increased by 151% compared to the pre-labeling sampling date a few days prior. Again, no overall effect of irrigation was noted in the general model (Table 3.1).

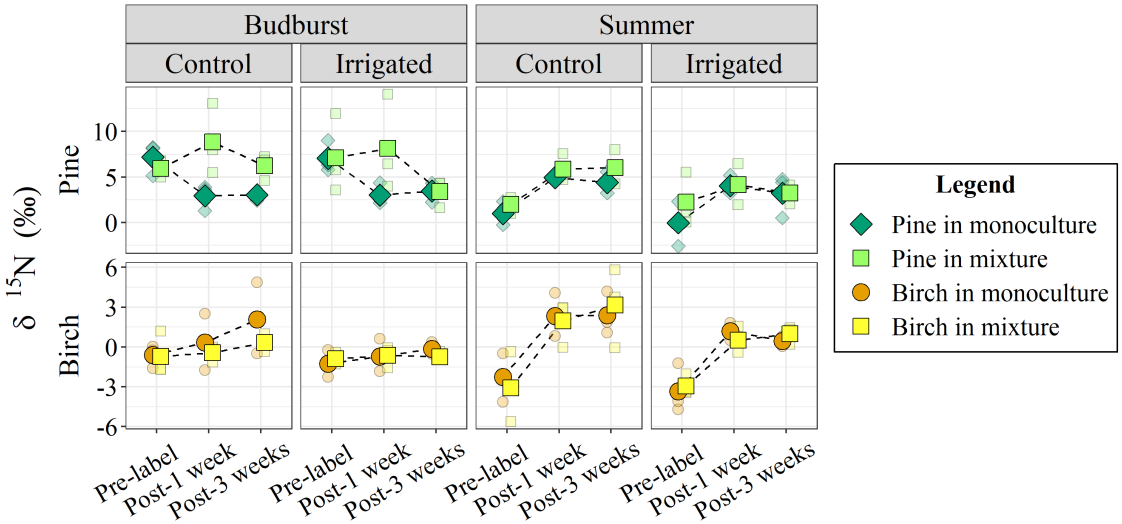

Figure 3.5: Values of pine needle (top) and birch leaf (bottom) $\delta^{15}N$ prior to labelling, 1 week after labelling, and 3 weeks after labelling, for the budburst (March 16th) and summer (June 8th) 2020 soil labeling campaigns. Pre-labeling sampling for the budburst campaign occurred in July 2019. Data are presented for pine in its monoculture (diamonds, green) and in the birch-pine mixture (squares, *light green*), and for birch in its monoculture (circles, *yellow*) and in the birch-pine mixture (squares, *light yellow*). At each leaf sampling date, the raw data are presented in partial translucence, with the mean value superposed (n = 3).

3.4 Discussion

3.4.1 No overall effect of mixing on soil nutrient availability

Contrary to our first hypothesis (H_1), we did not observe an increase in net nutrient mineralization or soil nutrient availability with mixing. The soil incubations using Raison's method to estimate net nutrient mineralization at various periods between early April and late September, showed no significant differences among species composition treatments (Fig. 3.4). Notably, the trends observed for net N mineralization were the same as for net P mineralization, suggesting a stable microbial N:P stoichiometry in our soils (Mooshammer et al. 2012), potentially due to similar microbial functioning (Fanin et al. 2013) despite

differences in species. In general and as expected, more nutrients were recovered during the growing season, due to the stimulation of microbial activity with the temperature increase at this time (Davidson et al. 2006). These fluctuations between dormant and growing season were especially observed in the control blocks (Fig. 3.3) subject to the dry summers of southwestern France (Fig. S3.1). Over the various six-month ion-exchange resin (IER) incubation periods, there was no effect of mixing on soil nutrient availability, in line with the neutral effect of mixing on soil mineralization observed. This may be because the IER used to determine nutrient availability captured nutrients that were either not immobilized by microorganisms, and not taken up by surrounding trees. Therefore, in order to further understand belowground dynamics in mixed-species plots, we investigated tree uptake at key phenological phases, budburst and peak summer vegetation.

3.4.2 Asynchrony of uptake at budburst, then lost at summer

In order to explore potential asynchrony of uptake between birch and pine in the mixture, soil labeling campaigns were done at budburst, and at peak leaf development in early summer. Prior to these campaigns, pine had a significantly higher ^{15}N isotopic signal than birch (Fig. 3.5), although both species had values consistent within the range of values for the mean annual temperature and precipitation of our experimental site (Craine et al. 2015b). The large difference could be attributed to differences in primary nutrient uptake depth, as $\delta^{15}N$ increases with depth (Craine et al. 2015a). This suggests that pine takes up nutrients on average from deeper soil layers and that nutrient uptake is not uniform along all roots, as pine and birch had similar root profiles at our site (Altinalmazis-Kondylis et al. 2020).

The labeling campaign at budburst revealed interesting patterns in the birch-pine mixture. At this stage, birch and pine behaved differently in the mixture: birch had a lower $\delta^{15}N$ while pine had a higher $\delta^{15}N$ than each of their respective monocultures. We cannot quantify whether this uptake is greater overall in the mixture than the expected mixing value because we did not calculate the quantity of label recovered using each tree's biomass; complete removal of the trees would have been required to calculate the complete quantity of ^{15}N absorbed (Bazot et al. 2016; Maxwell et al. 2020b). Nevertheless, the isotopic signal gives us information about the functioning of each species when mixed. At the current age and density, pine overtook birch for productivity in the mixture (Morin et al. 2020). After 12 years of growth, birch trees were largely overshadowed by pine (Table 3.2), which largely took up canopy space (Martin-Blangy et al. submitted). This was especially true in the low water availability treatment, where the positive mixing effect on the average tree crown volume was greater for pine than in the high water availability treatment, while the negative effect for birch was similar between the water treatments (Martin-Blangy et al.

submitted). The overshadowing by pine in the mixture led to a decrease in crown volume of birch, and may have also led to a delayed budburst for birch in the mixture compared to its monoculture, as previously found for oak mixed with pine (Perot et al. 2021). Both of these factors may explain why the birch trees had a smaller isotopic signal in the mixture than in the monoculture, i.e. the trees were less able to take up the soil-applied ^{15}N. While N can be absorbed by the tree prior to leaf swelling and budburst (Bazot et al. 2016; Maxwell et al. 2020b), pine trees had the advantage of a full canopy cover that included the previous cohorts' needles; in general, conifers tend to start transpiration and nutrient uptake prior to the leaf growth of broadleaf trees (Rothe 1997). Interestingly, pine in the mixture took up more than in the monoculture, indicating that it took advantage of birch's later budburst. This could indicate both a release of intraspecific competition for pine, in combination with an increase in interspecific competition for birch in the mixture – i.e. a win-lose situation (Forrester et al. 2011; Nanami et al. 2011; Vanclay et al. 2013). Pine's increase in uptake at the detriment of birch is unlikely due to differences in the understory biomass, as the latter was slightly higher in the mixture than in pure pine (Table 3.3). The specific dynamics between birch and pine, i.e. deciduous or coniferous trees, are thus closely connected to the stand structure (Rötzer et al. 2004), such as density, tree height, canopy cover, and understory biomass. We observed that these factors played an important role in determining uptake dynamics at budburst in the birch-pine mixture.

The summer labeling campaign resulted in similar uptake patterns for the two species in their monocultures and mixture (Fig. 3.5). At this period in early June, both trees had reached their maximum leaf canopy, and with it their maximum uptake capacities prior to the unfavorable dry conditions in peak summer (Gholz et al. 1990). With these results, we thus validate our second hypothesis (H_2), that asynchrony between the evergreen conifer and broadleaf deciduous would be strongest at budburst.

3.4.3 Effect of water availability on belowground processes

Contrary to our third hypothesis (H_3), belowground process rates did not accelerate with an increase in water availability. Over the growing period, irrigation tended to have a negative effect on net nutrient mineralization, indicating higher net microbial immobilization in these plots. This could be due to the higher competition between microbes and trees, as irrigation increased aboveground productivity (Table 3.2). As for tree nutrient uptake, the $\delta^{15}N$ signal was not stronger in irrigated blocks as there was no difference between water availability treatments. Although this is consistent with the late activation of the irrigation treatment in 2020 (one week prior to labeling), the lack of water treatment effect implies that there was not a strong legacy effect of reduced water availability on nutrient uptake. Contrary to previous studies finding an increased allocation to roots with drier conditions

(Bakker et al. 2006; Bello et al. 2019), our site did not exhibit differences in root biomass between control and irrigated blocks (Altinalmazis-Kondylis et al. 2020); this may explain why we observed no difference in uptake with water availability.

Additionally, there was a negative effect of irrigation on available mineral N and P, the latter being most affected by irrigation during the growing season (i.e. when irrigation was in effect; Fig. 3.3). Leaching (due to more precipitation with irrigation) may explain the overall negative effect of irrigation. The ion-exchange resins were placed 8 cm belowground, and a previous study at our site found that irrigation decreased available nutrients in the top 0-15 cm layer, but then increased nutrients down to 90 cm (Maxwell et al. 2020a). Nevertheless, interesting trends emerged among species composition treatments under improved water availability. Significantly higher mineral N was found in the pure pine than in either the pure birch or mixed birch-pine plots, and this was consistent over the two years of monitoring. The poor sandy soils of the experimental site ORPHEE's region have a widespread and severe limitation in phosphorus (Achat et al. 2013a; Trichet et al. 2008). The accumulation of N could be indicative that the pine trees in monocultures did not require all the available N. Pine trees in monocultures were around 2.5 times larger than the birch trees (Table 3.2), and thus they likely had higher rates of remobilization (Miller and Miller 1987), i.e. the ability to use stored internal N resources for growth and reproduction. As trees develop, the rate of N uptake slows concomitantly with an increase in their potential storage capacity (Miller 1986).

Meanwhile, pure birch trees under irrigation also displayed significantly higher available P than pure pine or the mixture during the growing season (Fig. 3.3B, Table S3.1). This is similar to a previous study on volcanic soils, which found that as precipitation increased, the effect of species was more relevant, with broad-leaved deciduous species exhibiting the highest values of P compared to conifers (Satti et al. 2007). Indeed, hardwood species, such as birch, are more effective at cycling nutrients, such as P, through foliage and litterfall back to the soil than softwood species, such as pine, due to the higher quality of their leaf litter (Achat et al. 2013a; Hobbie 2015; Sherman et al. 2006).

3.4.4 Synthesis and implications for nutrient cycling in mixed species forests

The three subsequent experiments (IER for soil nutrient availability, soil incubations for net nutrient mineralization, and soil ^{15}N labeling for uptake) gave us an overview of belowground nutrient cycling processes at different temporal resolutions when mixing birch and pine (Fig. 3.6). In the early spring, birch's later budburst within the mixture, led to temporal asynchrony of nutrient uptake, giving pine a competitive advantage for uptake (Fig. 3.6). Pine's increase in nutrient uptake in the mixture was due to birch's delayed budburst, likely due the much larger canopy space taken up by pine *vs.* birch (Martin-Blangy

et al. submitted; Morin et al. 2020). This mixing effect was lost at peak summer vegetation, when both species had reached their maximum of foliage development. There were also no differences in net nutrient mineralization among the different species composition (pure birch, pure pine, and birch-pine) throughout the growing season, implying that nutrient dynamics may not be constrained by microbial organic matter mineralization. Instead, differences in available nutrients among species composition may be more affected by nutrient needs and uptake patterns according to composition.

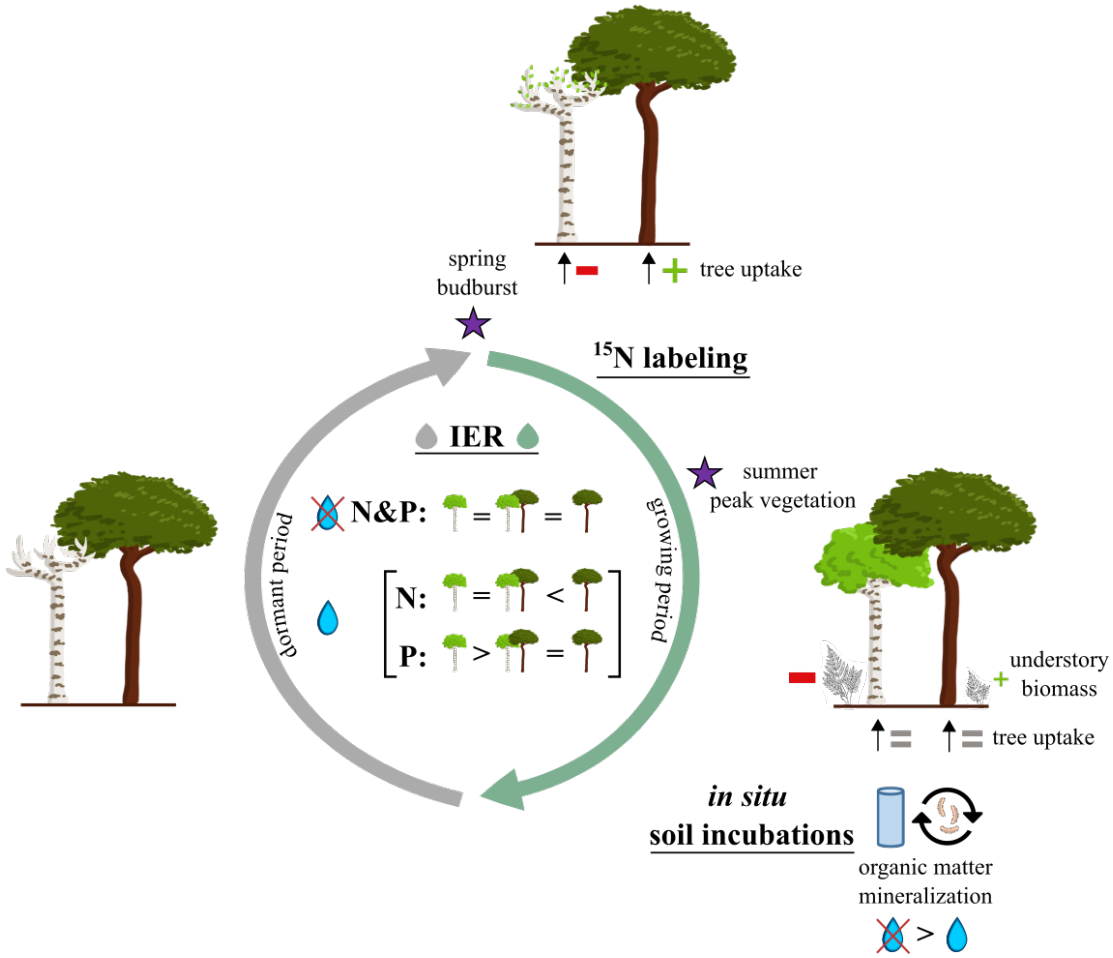

Figure 3.6: Belowground nutrient cycling at various periods throughout the year. (Top) From the spring budburst labeling campaign (*purple star*), birch took up fewer nutrients than its monoculture (*red negative symbol*) while pine took up relatively more than its monoculture (*green positive symbol*). (Bottom right) After the peak summer vegetation labeling campaign (*purple star*), both birch and pine in the mixture had similar uptake rates as in their respective monocultures. During summer, the mixture had greatly less understory than the pure birch plots (*red negative symbol*), slightly higher than pure pine (*green positive symbol*), and overall similar mineralization by soil microorganisms. Nutrient mineralization rates were overall higher in control than in irrigated plots. (Center) Under irrigation, more nitrogen (N) was available in pure pine than in pure birch and the mixture, while more phosphorus (P) was available in pure birch than either of the other two stands. The nutrient availability trends stayed constant from the growing to the dormant period.

The strong effect of mixing on uptake at budburst (negative for birch, positive for pine) was true under both water availability treatments, hence we suggest that species composition was more important than water availability to determine uptake patterns and more generally, belowground nutrient cycling, at our experimental site. Because broadleaf deciduous and evergreen conifers have different phenology patterns, potential temporal asynchrony in nutrient uptake is more likely at certain periods of the growing season. At these periods, such as budburst or in early autumn when leaves start to fall, differences in growth strategies may lead to potential asynchrony, increasing nutrient uptake in mixed species forests. Studies which further investigate the changes in timing of budburst of broadleaved species mixed with evergreen species, in concomitance with nutrient uptake measurements would contribute to the better understanding of potential temporal complementarity of belowground nutrient cycling in mixed species forests.

3.5 Conclusions

We investigated the potential interactive effect of mixing two tree species (birch and pine) and water availability on various belowground nutrient cycling processes using a unique experimental site in southwestern France. By looking into processes at various time points throughout the vegetation period, we hoped to elucidate potential temporal complementarity when mixing species with different growth strategies. We found that birch's delayed budburst in the mixture led to asynchrony in nutrient uptake, in a lose-win situation with pine which capitalized on birch's later budburst. Under high water availability, differences in soil nutrient availability throughout both the dormant and the growing season were revealed, due to differences in nutrient needs of the species. While we did not find differences in net mineralization between species, gross rates may have differed and may also contribute to explaining the differences in soil nutrient availability found between species under high water availability. Overall, we conclude that mixing a deciduous with an evergreen species can lead to asynchrony in nutrient uptake at budburst, but that no temporal complementarity on net mineralization was revealed. Increasing soil water availability can lead to differences in the influence of tree species on soil nutrient availability, due to differences among species in tree canopy cover and nutrient needs.

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3.6 Supplementary information

Table S3.1: Results of the mixed model analysis of the effect of species composition on soil nutrient availability, for each water availability treatment separately. F-values and p-values from the mixed model are presented. When species composition was a significant fixed effect, Tukey's HSD tests were done between species compositions (pure birch (Be), pure pine (Pp) and mixed Be-Pp). Significant ($p < 0.05$) and marginally significant ($p < 0.10$) effects are noted in bold font.

Variable	Irrigation	Fixed effect	F-value	p-value	Be vs Be-Pp	Pp vs Be-Pp	Pp vs Be
Mineral N ($N\text{-}NH_4^+$+ $N\text{-}NO_3^-$)	Control	Species	1.21	0.387	-	-	-
	Irrigated	Species	6.38	**0.057**	0.992	**0.009**	**0.005**
Mineral P ($P\text{-}PO_4^{3-}$)	Control	Species	0.03	0.969	-	-	-
	Irrigated	Species	6.27	**0.059**	**0.002**	0.488	**0.038**
$N\text{-}NH_4^+$	Control	Species	0.11	0.896	-	-	-
	Irrigated	Species	1.51	0.325	-	-	-
$N\text{-}NO3^-$	Control	Species	0.74	0.532	-	-	-
	Irrigated	Species	6.47	**0.056**	0.973	**0.005**	**0.008**

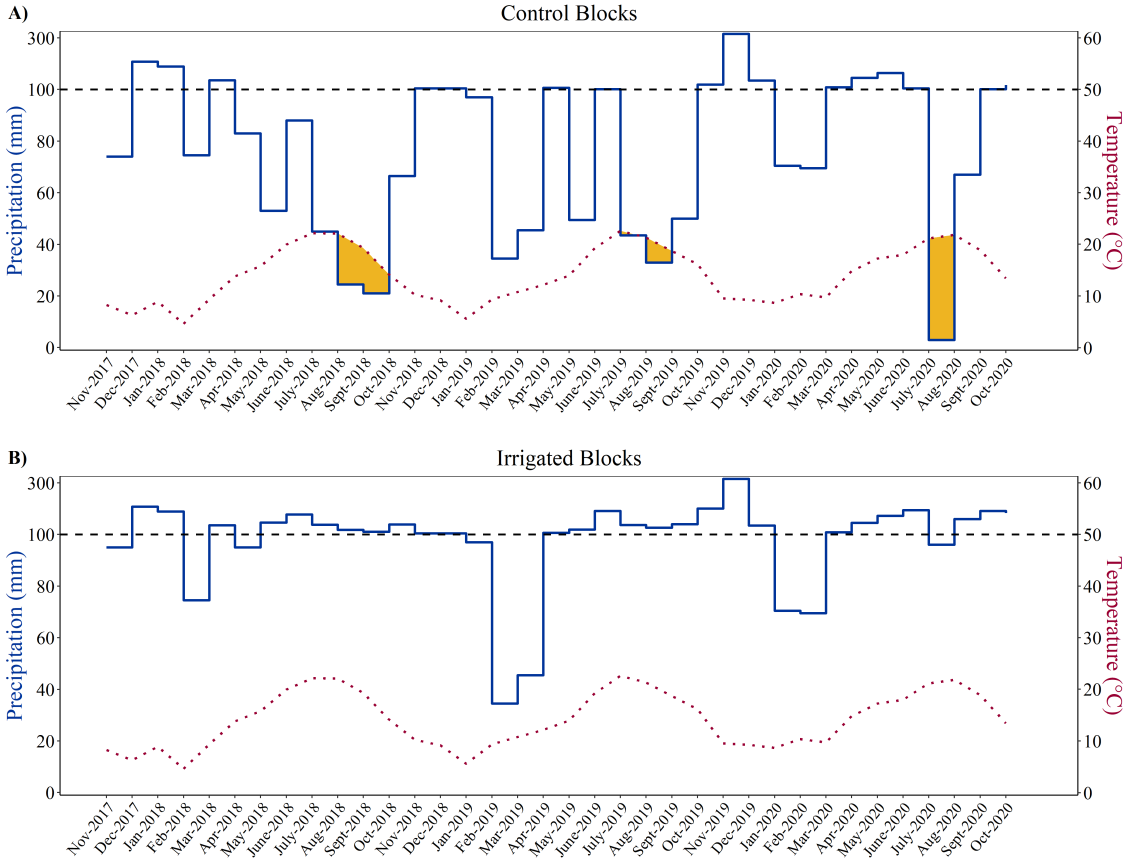

Figure S3.1: Ombrothermic diagram with total monthly precipitations (blue solid line) and average monthly temperature (red dotted line) during the study period for the control and irrigated treatments. Data were obtained from an INRAE weather station at Cestas Pierroton (44.742°N - 0.782°E), within 1 km of the experimental site. Temperature scale is set to 2mm/°C, and values above the 100mm black dashed horizontal line are scaled to 20mm/°C (Walter and Lieth 1967). When temperature is two times greater than precipitation, there is an arid period identified in yellow.

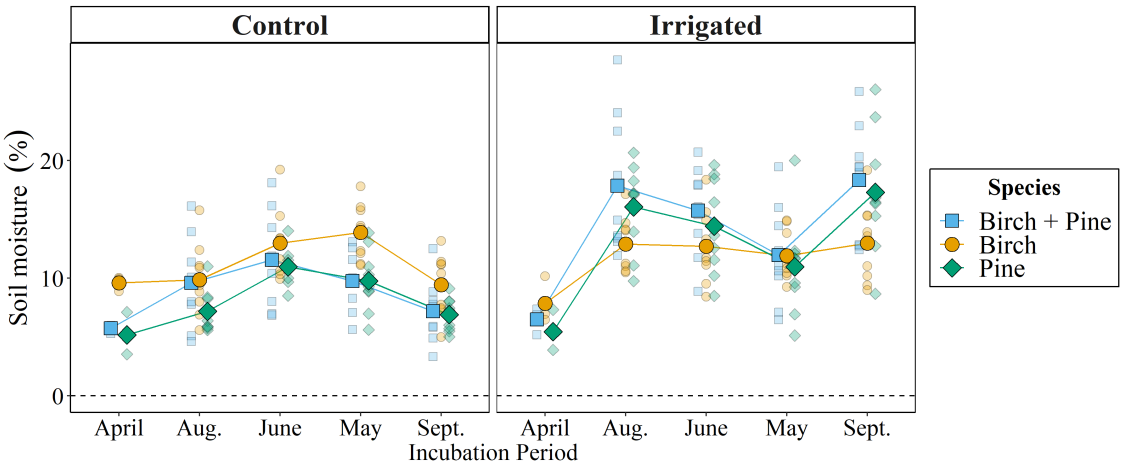

Figure S3.2: Soil moisture from uncovered soils recovered at each sampling date during Raison's soil incubation method in 2019. Values for mixed birch + pine (squares, *blue*), pure birch (circles, *yellow*), and pure pine plots (diamonds, *green*) are presented under both control and irrigated water availability treatments.

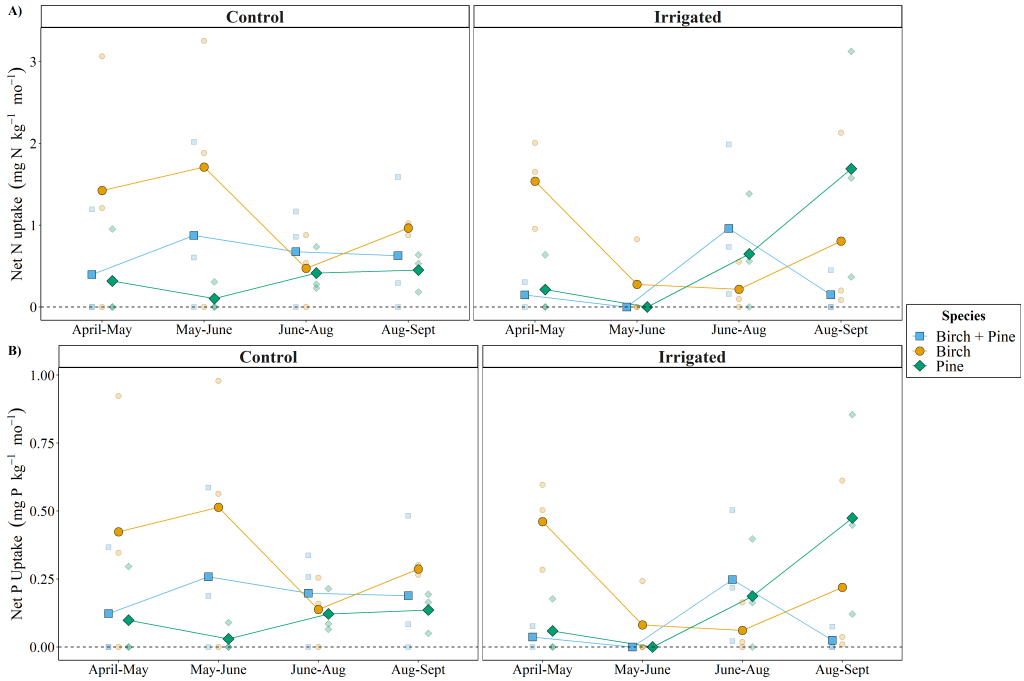

Figure S3.3: Net mineral N and P uptake in mixed birch + pine (squares, *blue*), pure birch (circles, *yellow*), and pure pine plots (diamonds, *green*) under both control and irrigated water availability treatments. At each incubation period in 2019, the raw data are presented in partial translucence, with the mean value superposed (n = 3).

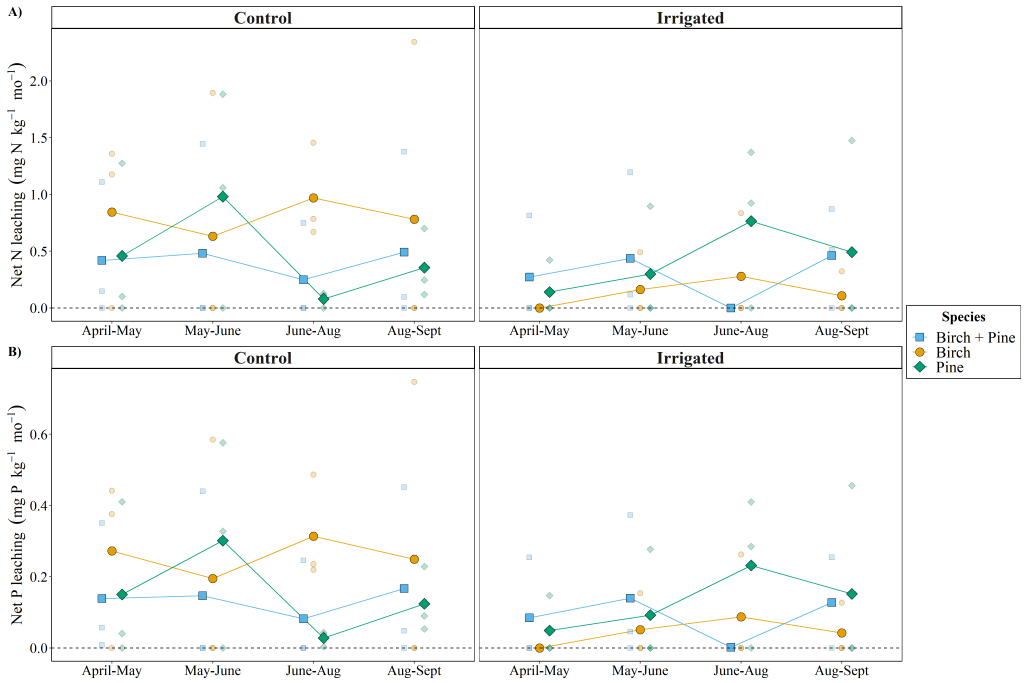

Figure S3.4: Net mineral N and P leaching in mixed birch + pine (squares, *blue*), pure birch (circles, *yellow*), and pure pine plots (diamonds, *green*) under both control and irrigated water availability treatments. At each incubation period in 2019, the raw data are presented in partial translucence, with the mean value superposed (n = 3).

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CHAPTER FOUR

Tree species dominance drives nutrient use efficiency in young mixed-species plantations, at both high and low water availability

Manuscript under review from Maxwell, T.L., Fanin, N., Parker, W., Bakker, M.R., Belleau, A., Meredieu, C., & Augusto, L., Munson, A.D.

Abstract

Previous studies have demonstrated that tree diversity increases productivity and may enhance nutrient cycling in forests. The effect of mixing tree species on stand-level nutrient use efficiency (NutUE) has seldom been studied, and even less so in the context of climate change. Here we present the first study examining how diversity effects on NutUE may be modified by seasonal changes in water availability, and importantly, during periodic drought or on water-limited sites. To do so, we tested how the interaction of water availability and tree diversity (i.e. species richness and species identity) affects NutUE in two plantation experiments, at ORPHEE in south-western France, and at IDENT-SSM in northern Ontario, Canada. We calculated NutUE as the aboveground net primary productivity (ANPP) of the stand divided by the flux of litterfall macronutrients of several tree species growing in monocultures or in mixtures (with a focus on birch and pine). We found significant species richness and water availability effects on NutUE, but they were weakly and inconsistently expressed, detected only for specific nutrients, and differed between the two sites. Species identity effects were more explanatory when examining the birch-pine plots at both sites. At ORPHEE, nitrogen use efficiency (NUE) and phosphorus use efficiency (PUE) were significantly greater in the pine-birch mixture than in each monoculture. This was due to an increase in ANPP together with a decrease in litter nutrient concentrations in the 2-species plot, driven by the dominance of pine in this mixture. In the younger and denser plots of IDENT-SSM, birch dominated the canopy space and led to both a positive effect of mixing on ANPP and litter nutrient concentrations in the birch-pine mixtures, leading to a neutral effect of mixing on NUE and PUE. Overall, the effects of mixing were similar in both of the contrasting water availability treatments (low *vs.* high), suggesting that species composition of mixtures is more important than water availability for stand-level NutUE in young forest communities.

Keywords: biodiversity, drought, litterfall, nutrient cycling, nutrient resorption efficiency, species identity, TreeDivNet

Résumé

Ce chapitre vise à étudier comment l'interaction entre la disponibilité en eau et la diversité des arbres affecte l'efficience d'utilisation des nutriments (NutUE) des arbres dans deux sites : ORPHEE, France et IDENT-SSM, Canada. La NutUE est la productivité primaire nette aérienne (ANPP) du peuplement, divisée par le flux de macronutriments de la chute de la litière. Les effets d'identité d'espèces se sont avérés plus explicatifs que l'effet de la richesse spécifique. À ORPHEE, l'efficacité d'utilisation de l'azote (NUE) et l'efficacité d'utilisation du phosphore (PUE) était significativement plus grande dans le mélange pin-bouleau que dans chaque monoculture, dû à la dominance du pin. Dans les parcelles plus jeunes et plus denses de IDENT-SSM, le bouleau a dominé l'espace de la canopée, conduisant à un effet neutre du mélange sur la NUE et la PUE. Dans l'ensemble, les effets du mélange étaient similaires dans les deux traitements de disponibilité d'eau.

Mots clés: biodiversité, chute de la litière, identité des espèces, efficacité de la résorption des nutriments, recyclage des nutriments, sécheresse,TreeDivNet

Chapter Introduction

Nutrient use efficiency

General context

The last step to complete our study of nutrient cycling in forests, is nutrient use efficiency (NutUE), i.e. the efficiency by which tree species utilize available nutrients to produce tree biomass (Binkley et al. 2004; Monteith 1977). Tree NutUE is calculated as aboveground net primary productivity (ANPP) divided by the total amount of nutrients in the litterfall (Vitousek 1982). Thus, a tree which produces more biomass per unit of nutrient uptake is considered to be more nutrient efficient (Fig. 4.1).

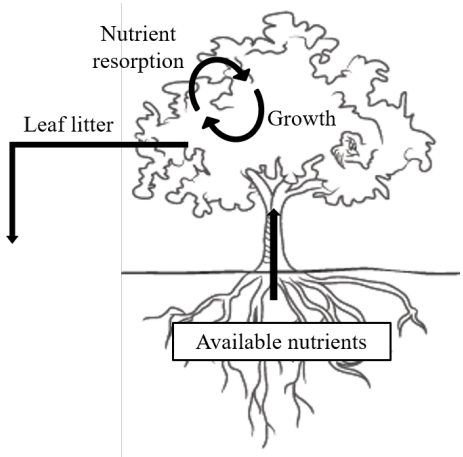

Figure 4.1: Processes influencing tree nutrient use efficiency (NutUE). NutUE is the division on aboveground net primary productivity by the litterfall nutrient flux. Litter nutrient concentration depend on nutrient resorption efficiency, which is influenced by the available nutrients.

Tree species richness and water availability can increase stand-level NutUE, by some combination of increasing ANPP, decreasing litter mass and/or decreasing litter nutrient concentrations (Binkley et al. 2004). Additionally, both tree species richness and water availability have been found to increase soil nutrient availability, which can have an indirect effect of decreasing NutUE by increasing litter nutrient concentrations due to a lower nutrient resorption efficiency (NutRE), i.e. the ability of trees to efficiently retranslocate foliar nutrients to developing tissues or storage sites prior to leaf abscission (Wright and Westoby 2003). However, the interactive effect of water availability and tree diversity on NutUE in forests is not well understood.

Objective and hypotheses

In the present chapter, we asked how water supply may influence the effect of species richness and species identity on NutUE at the stand-level. This study was conducted using temperate common gardens (ORPHEE, France and IDENT-SSM, Canada) established at two field experiments that manipulate both tree diversity and water availability during the growing season. We measured annual leaf litterfall mass production, green leaf and litter nutrient concentration, and ANPP to estimate stand-level NutUE at each site.

We hypothesized that ($H_{4.1}$) mixing tree species would decrease NutUE in comparison to monocultures, due to an increase in soil nutrient availability following from enhanced decomposition associated with more diverse leaf litter (Chapman et al. 2013; Handa et al. 2014; Talkner et al. 2009). Increased soil nutrient availability should in turn lead to lower retranslocation (Achat et al. 2018; Wright and Westoby 2003) and thus to an increase in litter nutrient concentrations. Because we expected increases in production in mixed-species plots *via* isometric growth between aboveground net primary productivity and annual litterfall mass (Enquist and Niklas 2002), the increase in litter nutrient concentration with mixing would lead to a decrease in NutUE with mixing.

We also hypothesized that ($H_{4.2}$) higher water availability would have a positive effect on NutUE due to higher growth (both ANPP and litterfall mass) per unit of nutrient.

Finally, because we expected a strong, positive diversity effect under low water supply on soil nutrient availability (Ratcliffe et al. 2017), we hypothesized that ($H_{4.3}$) the negative effect of mixing trees on NutUE would be stronger with decreasing water availability.

Experimental design

We measured annual leaf litterfall mass production, green leaf and litter nutrient concentration of selected macronutrients, and aboveground net primary productivity (ANPP) of several tree species growing in monocultures and in mixtures at the ORPHEE (Fig. 4.2) and IDENT-SSM experimental sites (Fig. 4.3). We then calculated stand-level nutrient use efficiency (NutUE) as ANPP divided by the flux of macronutrients induced by litterfall. We also estimated soil nutrient availability by installing ion-exchange resins, and calculated nutrient resorption efficiency using both green leaf and litter nutrient concentration, along with a mass leaf correction factor.

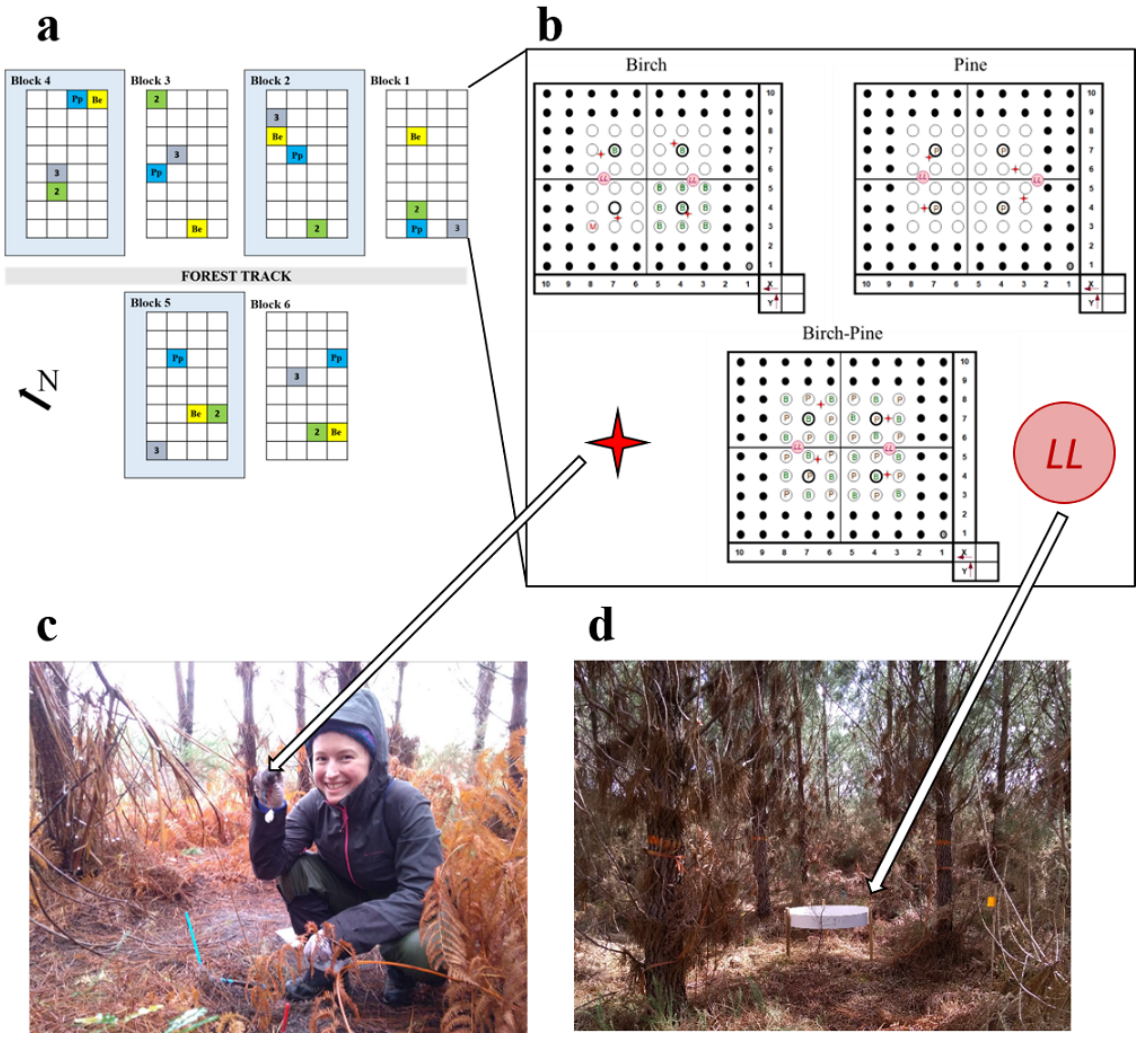

Figure 4.2: Experimental design at ORPHEE. (a) Six blocks sampled at ORPHEE, three control and three irrigated (*blue rectangles*), (b) Three species composition at the plot level (400 m^2) in each block, with the location of the two large litter traps (LL, *red circles*) and the four ion-exchange resins (*red stars*) per plot (example given for Block 1), (c) Recovering an ion-exchange resin in the fall, and (d) A large litter trap in a pure pine plot, hung aboveground by wooden stakes.

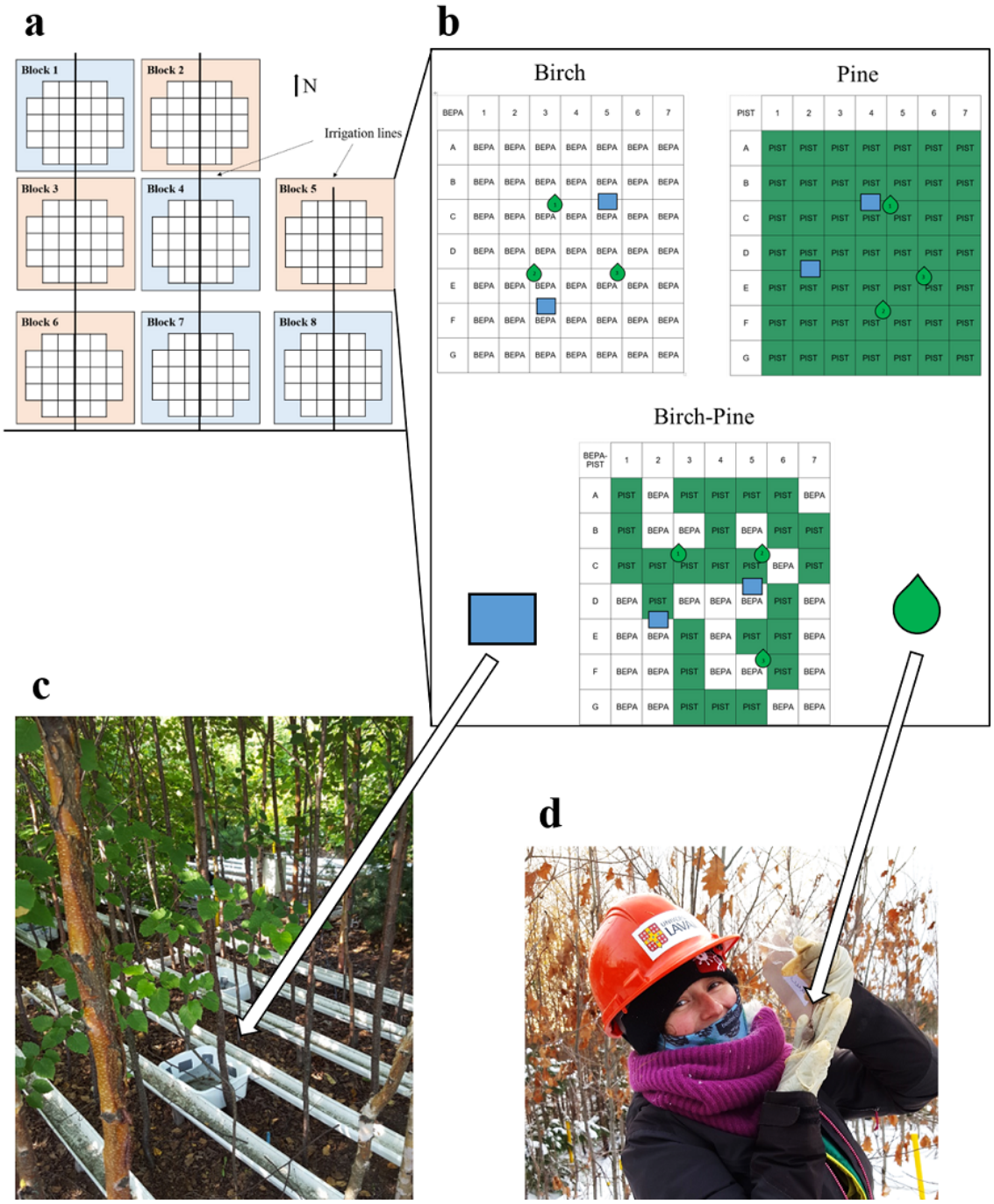

Figure 4.3: Experimental design at IDENT-SSM. (a) Eight blocks sampled at IDENT-SSM, four rain exclusion (*pink squares*) and four irrigated (*blue squares*), (b) Three of the species composition at the plot level (49 m^2) in each block, with the location of the two litter traps (*blue squares*) and the three ion-exchange resins (*green drops*) per plot (example given for Block 5), (c) A litter trap in a pure birch plot, hung by the gutters for rain exclusion, and (d) Recovering an ion-exchange resin in the fall.

Main results

We found significant species richness (SR) and water availability effects on NutUE, but they were weakly and inconsistently expressed (contrary to our general hypotheses $H_{4.1}$ & $H_{4.2}$), detected only for specific nutrients, and differed between the two experimental sites.

Species identity effects proved to be more explanatory when examining the birch-pine plots at both sites. At ORPHEE, nitrogen use efficiency (NUE) and phosphorus use efficiency (PUE) was significantly greater in the pine-birch mixture than in each monoculture. This was due to an increase in ANPP together with a decrease in litter nutrients in the 2-species plot, driven by the dominance of pine in this mixture. In the younger and denser plots of IDENT-SSM, birch dominated the canopy space and led to both a positive effect of mixing on ANPP and litter nutrient concentrations in the birch-pine mixtures, leading to a neutral effect of mixing on NUE and PUE.

Overall, the effects of mixing were similar in both the low and high water treatments (contrary to $H_{4.3}$), suggesting that species composition of mixtures is more important than water availability for stand-level NutUE in young forest communities.

Take-home message

Tree species composition (rather than species richness) is a stronger driver for nutrient use efficiency (NutUE) than water availability, and mixing certain tree species can be a viable way to improve stand-level NutUE in forests provided that the complementarity of species is correctly assessed.

4 Paper 4: Tree species dominance drives nutrient use efficiency in young mixed-species plantations, at both high and low water availability

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IDENT-SSM in May, August, and November 2019. Photos: T. L. Maxwell.

4.1 Introduction

Forests are increasingly impacted by global change, such as more intense and frequent drought periods (IPCC 2014; Lindner et al. 2014; Schwalm et al. 2012). Various adaptive management approaches have been suggested to mitigate these effects (Vilà-Cabrera et al. 2018). For example, increasing tree diversity is one long-term management strategy to increase resistance and resilience of forests to drought (Lebourgeois et al. 2013; Pretzsch et al. 2013b), due to asynchrony and complementarity (shade, nutrient content, water use) among species (Jucker et al. 2014a; Kardol et al. 2018; Morin et al. 2014). Although forests provide a multitude of ecosystem services (Jactel et al. 2017; Liang et al. 2016), tree productivity is often central to research questions. Tree productivity is tightly linked to nutrient use efficiency (NutUE), i.e. the efficiency by which tree species utilize available nutrients to produce tree biomass (Binkley et al. 2004; Monteith 1977). Tree NutUE is calculated as aboveground net primary productivity (ANPP) divided by the total amount of nutrients in the litterfall (Vitousek 1982). Thus, a tree which produces more biomass per unit of nutrient uptake is considered to be more nutrient efficient. Productive forests tend to have high nitrogen (NUE) and phosphorus (PUE) use efficiencies, due to changes in carbon allocation and nutrient mean residence times (Binkley et al. 2004; Richards et al. 2010). Tree productivity also depends on light and water availability, and their interaction with nutrient availability may determine the productivity of tree species when grown in mixtures (Richards et al. 2010). In the present study, we focused on the effect of tree diversity, expressed as species richness (SR) and species identity, and water availability on stand-level NutUE, which can be increased by some combination of increasing ANPP, decreasing litter mass and/or decreasing litter nutrient concentrations (Binkley et al. 2004).

Past studies frequently showed a positive effect of SR on litter mass production (Huang et al. 2017; Scherer-Lorenzen et al. 2007) and aboveground productivity (Chisholm et al. 2013; Liang et al. 2016; Morin et al. 2011; Piotto 2008; Toïgo et al. 2015; Zhang et al. 2012). The authors attributed this effect to an increase in structural heterogeneity (Pretzsch et al. 2016), or spatiotemporal complementarity in light interception (Pretzsch et al. 2014; Williams et al. 2017), related to differences in shade tolerance among species (Cordonnier et al. 2018a). Although the effect of light has been extensively studied during the last two decades, water availability can also affect the relationship between tree diversity and productivity (Belluau et al. 2021; Grossiord 2020). On water-limited sites, irrigation often increases both ANPP and litter mass of single species plantations (Campoe et al. 2013; Coyle et al. 2016). This is also true in mixed-species communities, as demonstrated in a recent global meta-analysis showing that overyielding increased with local precipitation (Jactel et al. 2018). Root stratification, hydraulic redistribution, or dominance of species with particular traits may all contribute to explain the positive effect of SR on aboveground biomass (Grossiord 2020). While trees in mixed forests are considered to be more resilient

to water shortage than those in monocultures (Ratcliffe et al. 2017), in general trees have been found to reduce ANPP and litter production under periodic drought conditions (Brando et al. 2008). Therefore, in addition to drought's negative effect on diffusive processes and the mobility of nutrients in the soil, one might expect that water-limited conditions may also decrease uptake and modify use of nutrients by trees (Schlesinger et al. 2016). However, the interactive effect of water availability and diversity on NutUE in forests is not well understood.

Tree diversity and water availability may influence stand NutUE through interactive effects on soil nutrient availability, nutrient resorption efficiency (NutRE), and litterfall nutrient concentrations (Richards et al. 2010). Independent of site moisture conditions, improved litterfall quality in mixed forests could accelerate litter decomposition, while increasing litter quantity could contribute to higher total input of N and P to the soil (Chapman et al. 2013; Handa et al. 2014; Talkner et al. 2009). Soil moisture deficits can decrease belowground process rates by reducing microorganism activity (Borken and Matzner 2009), which may decrease soil nutrient availability. Nutrient use efficiency is also partly influenced by NutRE (Hayes et al. 2014), i.e. the ability of trees to efficiently retranslocate foliar nutrients to developing tissues or storage sites prior to leaf abscission (Wright and Westoby 2003). When soil nutrient availability is high, fewer nutrients need to be remobilized from the green leaves (Achat et al. 2018; Wright and Westoby 2003); this can result in increased litterfall nutrient concentrations and decreased NutUE (Hayes et al. 2014). Studies investigating the direct effect of tree diversity on litterfall nutrient concentrations showed a positive (Huang et al. 2017; Xiao et al. 2019), neutral (Scherer-Lorenzen et al. 2007), or variable effect, depending on species identity of mixed species communities (Ball et al. 2008). While irrigation was found to have no effect on litterfall nutrient concentrations (Matías et al. 2011), changes in water availability can influence nutrient uptake, aboveground nutrient cycling or retranslocation (Schlesinger et al. 2016). To our knowledge, ours is the first study investigating the interactive effect of tree diversity and water availability on soil nutrient availability, NutUE, and NutRE.

The species identity of a mixture may be as important in determining diversity effects at the stand-level as SR (Baeten et al. 2019; Cordonnier et al. 2018b; Van Der Plas et al. 2016). Positive effects of mixing can depend on whether species interactions reduce competition for a growth-limiting resource (Forrester 2014; Forrester et al. 2006). For example, it has been previously observed that mixing species with different life-history strategies can lead to positive diversity effects (Grossiord 2020; Kelty 2006; Roscher et al. 2012). For example, conifer-deciduous admixtures have been found to be more resilient to drought than in mixtures of deciduous species (Pardos et al. 2021). This could be explained by the fact that evergreen coniferous species are resource conservative, having evolved in nutrient poor and xeric ecosystems, and are characterized by higher NutUE and lower litter nutrient

concentrations than deciduous species (Augusto et al. 2014; Berendse and Scheffer 2009; Chapin et al. 1993; Pastor et al. 1984). Identifying species with complementary nutrient use strategies for use in plantations on nutrient limited sites is also of great interest (Zeugin et al. 2010).

In the present study, we asked how water supply may influence the effect of species richness and species identity on NutUE at the stand-level. This study was conducted using temperate common gardens established at two field experiments that manipulate both tree diversity and water availability during the growing season. We measured annual leaf litterfall mass production, green leaf and litter nutrient concentration, and ANPP to estimate NutUE at each site. We hypothesized that (H_1) mixing tree species would decrease NutUE in comparison with monocultures, due to an increase in soil nutrient availability following from faster decomposition associated with more diverse leaf litter (Chapman et al. 2013; Handa et al. 2014; Talkner et al. 2009). Increased soil nutrient availability should in turn lead to lower retranslocation (Achat et al. 2018; Wright and Westoby 2003) and thus to an increase in litter nutrient concentrations. Because we expected increases in production in mixed-species plots *via* isometric growth between aboveground net primary productivity and annual litterfall mass (Enquist and Niklas 2002), the increase in litter nutrient concentration with mixing would lead to a decrease in NutUE with mixing. We also hypothesized that (H_2) higher water availability would have a positive effect on NutUE due to faster growth (both ANPP and litterfall mass) per unit of nutrient. Finally, because we expected a strong, positive diversity effect under low water supply on soil nutrient availability (Ratcliffe et al. 2017), we hypothesized that (H_3) the negative effect of mixing trees on NutUE would be stronger with decreasing water availability.

4.2 Materials and methods

4.2.1 Experimental Sites

Our study was conducted at two similar experimental sites located in temperate forest regions in France and in Canada, both of which are part of the worldwide Tree Diversity Network (TreeDivNet). These two common garden experiments (Table S4.1) were established to investigate the interaction of tree species diversity (hereafter referred to as *SR*, for species richness) and water availability (hereafter referred to as H_20 in several figures and tables) on ecosystem functions. Both sites have a low and high water availability treatment created by some combination of rainfall exclusion and/or irrigation.

The ORPHEE experiment is located in southwestern France (44°44.35’ N, 00°47.9’ W, elevation of 60 m asl), 40 km southwest of Bordeaux (Castagneyrol et al. 2013). The mean annual temperature is 12.5°C and the mean annual precipitation is 870 mm (1993 - 2018).

Most precipitation occurs from the fall to the spring, leading to climatic moisture deficits during the summer months. The predominant soil type is podzol, characterized by a rapid drainage, coarse texture (3% clay, 2% silt, 95% sand) and low fertility (Augusto et al. 2010), particularly in P (Trichet et al. 2009). The ORPHEE study was established in 2008, after clear-cutting of a *Pinus pinaster* stand, followed by a single ploughing and fertilizer application of 52.4 kg P ha^{-1} and 99.6 kg K ha^{-1}. A total of 256 plots were then planted, with five species (*Betula pendula* Roth. (Be), *Pinus pinaster* Ait. (Pp), *Quercus ilex* L., *Quercus pyrenaica* Willd., *Quercus robur* L. (Qo) in all possible combinations of one to five species mixtures. Each plot had 100 trees planted at 2 m spacing within a 20 m by 20 m plot area. The plots were separated by 3 m from one another, and randomly located within six blocks: three unirrigated, control blocks, subject to the dry summers, and three irrigated blocks. We selected three levels of SR to examine tree diversity effects: pure silver birch (Be), pure maritime pine (Pp), birch-pine mixture (Be-Pp), and pine-birch-oak mixture (Be-Pp-Qo). Plantations containing pine and birch were selected for the study because, contrary to the other tree species, they grew rapidly despite the low fertility of the sandy soil, resulting in relatively dense plots. Seasonal irrigation treatment began in 2015, to alleviate summer plant water stress from early May to late October. A two-meter-tall sprinkler installed in the center of each experimental plot delivered 3 mm every evening (equivalent to 21 mm per week) during this period. In the 2019 water treatment months (May 9th – October 28th), control blocks received 396 mm of precipitation, while irrigated blocks received a total of 912 mm of water (precipitation + irrigation) during the same period. Ombrothermic diagrams (Walter and Lieth 1967) for the study site during 2019 indicated control blocks were subjected to comparatively greater climatic moisture deficits than the irrigated blocks (Fig. S4.1).

The second site, IDENT-SSM, is located in Sault Ste. Marie, northern Ontario, Canada (46°32.47' N, -84°27.20' W, elevation of 210 m asl), and is part of the International Diversity Experiment Network with Trees (IDENT) (Belluau et al. 2021). The mean annual temperature is 4.7°C and the mean annual precipitation is 899 mm, which is evenly distributed throughout the year (1981 - 2020). The experiment was established in 2013 on a former low-input agriculture field of low soil fertility and water holding capacity. The soil texture varies from sandy loam to loamy fine sand, with an average of 8% clay, 12% silt and 80% sand. The area was treated with herbicide and ploughed in summer of 2012 in preparation for planting. Based on preliminary testing of soil characteristics, the area was limed with calcitic limestone (3 t ha^{-1}) in April 2013, and immediately after planting in May 2013, fertilized with a slow release NPK fertilizer (Nutricote®14-14-14, 1.5 t ha^{-1}). A total of 8,624 trees were planted in 176, 10.24 m^2 square plots that contained 49 trees arranged in 7 rows of 7 trees planted at 40 cm spacing using the IDENT experimental design (Belluau et al. 2021). Each plot represents one of 22 different 1, 2, 4 or 6 species mixtures composed of six native species (*Acer saccharum* Marsh (As), *Betula papyrifera*

Marsh. (Ba), *Larix laricina* (Du Roi) K. Koch (Ll), *Picea glauca* (Moench) Voss (Pg), *Pinus strobus* L. (Ps), *Quercus rubra* L. (Qu)). We chose to work with five monocultures (As, Ba, Ll, Ps, Qu), four 2-species mixtures (Ba-Ps, Ba-As, Ba-Ll, Ll-Qu), and two 4-species mixtures (As-Ll-Ps-Qu, As-Ba-Ll-Ps). Each mixture is replicated four times with two blocks exposed to high or low, growing season water treatments that commenced in June 2014. The high water treatment is created by weekly irrigation of the equivalent of 25.4 mm of rain (double the average quantity of weekly precipitation) over the course of four hours from June 1 – August 31. The other four blocks have a rain exclusion system that removes 25% of ambient throughfall precipitation from all plots from early May to late October (see Belluau et al. 2021 for further details). It rained a total of 205 mm during the summer months in 2019 (early June to late August): rain exclusion blocks intercepted 150 mm of precipitation, while irrigated blocks received 321 mm of water during the same period, resulting in a soil moisture deficit (Fig. S4.1).

Figure 4.4: Images of the birch-pine mixtures at the A) ORPHEE (*Betula pendula – Pinus pinaster*) and B) IDENT-SSM (*Betula papyrifera – Pinus strobus*) study sites in 2019. PC: T.L. Maxwell.

Both sites have the tree species combinations of pure birch, pure pine, and mixed birch-pine. Both pine species (*Pinus pinaster* and *Pinus strobus*) are calcifuge, with a preference for well-drained or sandy soils, and which establish at the early succession stage. Similarly, both birch species (*Betula pendula* and *Betula papyrifera*) are comparable deciduous species, as they are pioneer species and relatively short-lived, preferring mesic to slightly xeric sites and siliceous soils.

4.2.2 Ion-exchange resins

Ion-exchange resin (IER) capsules (UNIBEST, PST-1 Capsule) were installed at both sites to estimate annual soil nutrient availability (Binkley and Matson 1983). The capsules were placed at a depth of 8 cm in the topsoil for six months, retrieved, and replaced at a nearby location for a second 6-month period. The sum of the two consecutive 6 month IER samples provided an estimate of annual available soil nutrient concentrations. In ORPHEE, four IER capsules were installed in each plot from November 2018 – November 2019. The IER were extracted by 1M KCl and the final solution extractant was measured for ammonium (NH_4), nitrate (NO_3), and phosphate (PO_4) concentrations by colorimetry (SKALAR, San ++). At IDENT-SSM, three IER capsules were installed per plot from May 2019 to May 2020. The IER were extracted by 1M KCl and NH_4, NO_3 and NO_2 concentrations was measured by flow injection analysis (Lachat, FIA Quickchem serie 2) and the PO_4 concentration was measured by spectrometry (Agilent Technologies, ICP-OES 5110). The plots with tree communities that contained Qu (Qu, Ll-Qu, As-Ll-Ps-Qu) were not sampled.

4.2.3 Leaf sample collection and analyses

Litterfall mass was measured by collecting leaf litterfall several times a year in litterfall traps. At ORPHEE, the traps were made of a hollow, 10 cm tall plastic cylinder with a surface area of 0.72 m^2 with a mesh covering at the bottom, and hung on wooden sticks at 50 cm aboveground. Two litterfall traps were placed in the monocultures and 2-species mixtures, and three litterfall traps were placed in the 3-species mixture. Litterfall traps were already installed prior to the 2019 growing season, and litterfall was collected in May, June, August, October, December 2019 and February 2020. After each collection, samples were dried at 40°C to constant mass, separated by species, and weighed. Annual litterfall mass was calculated in g m^{-2} for each target species and plot. At IDENT-SSM, the litterfall traps were made of suspended, rectangular plastic basins about 27.5 cm by 32.5 cm in area (surface area of 0.09 m^2) and 14 cm height. The bottom of the basins had several 1.2 cm diameter holes drilled in them and were lined with window screening to allow rain water to drain out without loss of litterfall. Two litterfall traps per plot were

installed in May 2019, and the litterfall was collected in August 2019, October 2019, and May 2020. The litter was sorted by species, dried at 60°C for at least 48 hrs, and weighed to calculate annual litter mass for each species and plot. Oven dried litter was ground in preparation for nutrient analysis.

Green leaf collection occurred during the seasonal period of peak leaf development at both sites. At ORPHEE, 30 green leaves were collected in the upper canopy of four representative trees per species in each plot in July 2019. The composite leaf samples for each species and plot were dried at 40°C for at least 48 hrs. At IDENT-SSM, composite samples were collected in early August 2019 and consisted of 5-10 green leaves harvested along a diagonal plot transect from five trees per species in monocultures and 2-species mixtures, and three trees per species in the 4-species mixtures. Leaves were dried in an oven at 60°C for at least 48 hrs, separated by species and plot. Samples for both sites were ground and stored for subsequent nutrient analysis.

Both litter and green leaf samples from ORPHEE and IDENT-SSM were analyzed for N using an analyzer based on Dynamic Flash Combustion technology (Thermo Flash, EA 1112). A subset of ground leaves from ORPHEE was mineralized in an oven at 370°C for 4 hrs (J.P SELECTA, BLOC DIGEST M40). Afterwards, 75 ml of distilled water was added to the ashes, and the solution was analyzed for phosphorus (SEAL, AutoAnalyzer 3 HR), while potassium (K), calcium (Ca), magnesium (Mg) and manganese (Mn) were measured by flame atomic absorption spectrophotometry (VARIAN, SpectrAA-20). At IDENT-SSM, subsets of ground leaves were mineralized in an oven at 550°C during 6 hours, after which the ashes were placed in solution with $HCl : HNO_3$, which was then diluted and filtered. The samples were analyzed for P, K, Ca, Mg and Mn concentrations (Agilent Technologies, ICP-OES 5110).

4.2.4 Aboveground net primary productivity (ANPP)

Annual tree size assessments at both sites were used to estimate ANPP (kg m^{-2} yr^{-1}) for the 2019 growing season using allometric equations. At ORPHEE, tree diameter at 1.3 m above ground for all living trees within the central core area of the plot (i.e. excluding two perimeter rows of trees) and allometric equations for Pp (Shaiek et al. 2011) and Be (Johansson 1999) were used to calculate plot-level biomass by species. Qo biomass was negligible in the three-species plot due to poor growth of the species, and was not included in the plot-level ANPP and NutUE calculations. At IDENT-SSM, species-specific allometric equations to predict above-ground biomass from basal stem diameter were derived from annual destructive harvest of either potted seedlings or block-level buffer trees (Belluau et al. 2021). Only the central 25 trees were included in plot-level biomass estimation (i.e. excluding one perimeter row of trees). The difference in total plot and

species biomass between 2018 and 2019 was used as an estimate of ANPP.

4.2.5 Calculations

Nutrient use efficiency (NutUE) for each species in each plot for each nutrient was calculated using the following equation (Binkley et al. 2004):

$$NutUE = \frac{ANPP}{litterfall\ mass * litter\ nutrient\ concentration} / 1000 \tag{4.1}$$

where aboveground net primary productivity (ANPP) is measured in kg m^{-2} yr^{-1}, litterfall mass in g m^{-2} yr^{-1} and litter nutrient concentrations in mg g^{-1}, which is divided by 1000 to give NutUE values in g mg^{-1} nutrient. For mixed-species plots (i.e. a plot of species A and B), the NutUE calculation for each nutrient was the following:

$$NutUE = \frac{ANPP_A + ANPP_B}{(litterfall\ mass_A * litter\ nutrient_A) + (litterfall\ mass_B * litter\ nutrient_B)} \tag{4.2}$$

We also calculated the nutrient resorption efficiency (NutRE) for each species, plot and nutrient with the following equation (Augusto et al. 2017; Vergutz et al. 2012):

$$NutRE = \frac{([Nut]_{green} - ([Nut]_{scenescent} * MLCF))}{[Nut]_{green}} \tag{4.3}$$

where $[Nut]_{green}$ and $[Nut]_{senescent}$ are nutrient concentration values in green leaves and senescent leaves, respectively, and MLCF is the mass leaf correction factor used to account for the decrease in leaf mass during senescence (Vergutz et al. 2012). To calculate MLCF at IDENT-SSM, a subsample (n = 40-100) of equal numbers of oven-dried green and senescent (i.e. litter) foliage for a given species and plot, were weighed to estimate an average MLCF for each species, as the ratio of senescent to green leaf dry mass (pine = 0.761, birch = 0.582, larch = 0.732, maple = 0.519, oak = 0.548). At ORPHEE, where trees are older and taller than at IDENT-SSM, leaf mass varied widely along the vertical position in the canopy and prevented calculation of site-level MLCF values. Instead, values reported in literature (pine = 0.775, birch = 0.719) were used ((Vergutz et al. 2012). Comparatively low NutRE values imply decreased nutrient resorption and reflective of low site nutritional limitation (Augusto et al. 2017).

To estimate plot-level litterfall nutrient concentrations and NutRE for mixed-species plots, we first calculated the proportional contribution (*Prop*) of each species to the total litter mass (Eq. 4.4, 4.5). For example, in a 2-species mixture with species A and B:

$$Prop_{A\ in\ A+B} = \frac{litterfall\ mass_{A\ in\ A+B}}{litterfall\ mass_{A+B}} \tag{4.4}$$

$$Prop_{B\ in\ A+B} = \frac{litterfall\ mass_{B\ in\ A+B}}{litterfall\ mass_{A+B}} \tag{4.5}$$

These proportions were used to estimate the plot-level nutrient concentration $[Nut]$ (Eq. 4.6). The plot-level nutrient concentration $[Nut]$ for a plot with species A and B can be calculated by adding the product of each species nutrient concentration and litter mass proportion in the mixture:

$$[Nut]_{A+B} = [Nut]_A * Prop_{A\ in\ A+B} + [Nut]_B * Prop_{B\ in\ A+B} \tag{4.6}$$

This same equation was used to calculate plot-level NutRE for species mixtures by substituting NutRE for $[Nut]$.

4.2.6 Statistical Analyses

All of the statistical analyses were performed using R software (version 4.1.0). Prior to formal analysis, extreme values were removed from the data set following the outlier labelling rule with a conservative tuning parameter of g = 2.2 (Hoaglin and Iglewicz 1987). On average, from 0% (ANPP, litter mass, NutUE) to 4% (litter and soil nutrient concentrations) of data were removed. Mixed models were used to quantify the effect of the following fixed effect factors: SR, water availability treatment (H_2O), and their interaction on soil nutrient availability, ANPP, litter mass, litter [N], NutRE, and NutUE values separately at each site. Pairs of low and high H_2O blocks (i.e. one 'Superblock') were included as a random factor to account for the spatial structure of our experimental design, with (H_2O treatment nested into 'Superblock'. The models were coded using the '*nlme*' package (Pinheiro and Bates 2020, October 24) with α = 0.05 as significance level. Variables were log-transformed if needed to improve the normality of residuals. When significant fixed factor effects were found, a Tukey's HSD post-hoc test was used for treatment mean comparisons using the '*multcomp*' package (Hothorn et al. 2020, November 14). The same models were used to examine the species identity effects at each site, by replacing SR with 'species composition' (pine, birch, or pine-birch mixture).

Linear correlations between soil nutrient availability and NutUE values were determined with Pearson's product-moment correlation coefficients. Significant differences between the expected and observed values for each species in the mixed species plots in both H_2O treatments were determined with Welch two sample t-tests. These tests were also

used to test significant differences in green leaf nutrient concentrations between species and between H_2O treatments for each species. Graphs were generated with the '*ggplot2*' package (Wickham 2016).

Table 4.1: Results of mixed model analysis of species richness (SR), water availability treatment (H_2O) and their interaction on soil nutrient availability, productivity, and nutrient relations at the IDENT-SSM and ORPHEE sites. F-values and p-values for fixed effects are presented. Significant ($p < 0.05$) effects are noted in bold font. Abbreviations as defined earlier.

		SR		H_2O		SR × H_2O	
		F-value	p-value	F-value	p-value	F-value	p-value
IDENT-SSM	NH_4^+	0.632	0.536	2.319	0.225	1.084	0.346
	NO_3^-	1.206	0.308	4.177	0.134	0.558	0.576
	PO_4^{3-}	2.216	0.119	0.871	0.419	0.646	0.528
	ANPP	10.859	**<0.001**	3.969	0.140	1.121	0.331
	Litterfall mass	14.098	**<0.001**	7.224	0.075	2.432	0.095
	Litter N	10.249	**<0.001**	1.254	0.344	1.034	0.361
	Litter P	11.670	**<0.001**	2.144	0.239	0.419	0.659
	Litter K	1.911	0.155	1.284	0.340	0.761	0.471
	Litter Ca	0.954	0.390	0.017	0.903	0.383	0.683
	Litter Mg	7.537	**0.001**	79.553	**0.003**	0.011	0.990
	Litter Mn	0.794	0.456	11.115	**0.045**	0.257	0.774
	NUE	0.491	0.614	0.300	0.622	1.728	0.185
	PUE	1.278	0.285	0.003	0.963	0.946	0.393
	KUE	0.816	0.446	2.122	0.241	0.908	0.408
	CaUE	2.057	0.135	0.809	0.435	0.454	0.637
	MgUE	0.063	0.939	26.542	**0.014**	0.430	0.652
	MnUE	2.484	0.090	6.029	0.091	1.228	0.299
ORPHEE	NH_4^+	0.571	0.578	11.483	0.077	1.396	0.280
	NO_3^-	0.936	0.415	11.034	0.080	1.638	0.230
	PO_4^{3-}	0.553	0.587	8.829	0.097	0.638	0.543
	ANPP	3.379	0.063	2.922	0.230	0.615	0.555
	Litterfall mass	0.850	0.449	2.358	0.264	0.093	0.912
	Litter N	2.098	0.160	0.002	0.973	0.153	0.859
	Litter P	4.334	**0.034**	0.645	0.506	0.163	0.851
	Litter K	4.173	**0.038**	3.180	0.216	0.036	0.964
	Litter Ca	1.509	0.255	28.237	**0.034**	1.581	0.240
	Litter Mg	1.861	0.192	0.762	0.475	0.140	0.870
	Litter Mn	1.901	0.186	14.703	0.062	0.419	0.666
	NUE	4.196	**0.037**	0.230	0.679	1.201	0.330
	PUE	7.315	**0.007**	0.033	0.872	1.667	0.224
	KUE	9.052	**0.003**	0.774	0.472	2.140	0.155
	CaUE	1.886	0.188	6.413	0.127	4.440	**0.032**
	MgUE	4.544	**0.030**	0.049	0.845	1.502	0.256
	MnUE	3.993	**0.042**	8.793	0.097	1.308	0.302

4.3 Results

4.3.1 Soil and leaf nutrient concentrations

Available soil NH_4, NO_3, and PO_4 concentrations did not differ with SR, water availability treatment (H_2O) at either site and there was no significant interaction between SR and H_2O (Table 4.1, 4.2). At ORPHEE, there was a strong trend toward a negative effect of high H_2O on soil NH_4 ($p = 0.077$), NO_3 ($p = 0.080$), and PO_4 ($p = 0.097$) (Table 4.1, 4.2). At IDENT-SSM, although no significant differences were found between the rain exclusion and irrigated plots, high H_2O tended to result in higher availability of all soil nutrients (Table 4.2).

Table 4.2: Mean (± standard error) soil $N-NH_4^+$, $N-NO_3^-$ and $P-PO_4^{3-}$ concentrations and sample size (n) for species richness (SR) and water availability (H_2O) treatments at the ORPHEE and IDENT-SSM sites. Means for H_2O treatments across SR levels at a given site are presented in bold.

				N-NH_4^+		N-NO_3^-		P-PO_4^{3-}
Site	H_2O	SR	n	(µg capsule yr^{-1})	n	(µg capsule yr^{-1})	n	(µg capsule yr^{-1})
IDENT-SSM	Low	1	46	77.4 ± 12.0	46	9.4 ± 2.0	46	9.1 ± 1.7
	Low	2	35	93.7 ± 14.4	35	9.4 ± 1.9	35	11.1 ± 2.0
	Low	4	12	101.3 ± 28.3	12	12.5 ± 3.3	12	20.7 ± 7.0
			93	**86.6 ± 8.8**	**93**	**9.8 ± 1.3**	**93**	**11.4 ± 1.5**
	High	1	46	108.4 ± 14.5	48	15.2 ± 2.5	45	11.9 ± 1.8
	High	2	35	88.6 ± 15.1	36	14.4 ± 2.5	35	15.7 ± 2.5
	High	4	12	116.2 ± 23.4	12	11.7 ± 2.1	12	14.0 ± 3.0
			93	**102.0 ± 9.6**	**96**	**14.5 ± 1.6**	**92**	**13.6 ± 1.4**
ORPHEE	Low	1	20	99.5 ± 35.2	22	242.0 ± 53.3	24	37.3 ± 6.5
	Low	2	12	89.1 ± 27.1	12	207.2 ± 50.7	12	30.1 ± 7.0
	Low	3	9	129.9 ± 59.0	9	177.5 ± 74.3	9	21.0 ± 8.3
			41	**103.2 ± 22.5**	**43**	**218.8 ± 33.9**	**45**	**32.1 ± 4.3**
	High	1	18	3.4 ± 0.6	18	110.2 ± 28.2	18	10.0 ± 1.3
	High	2	10	2.3 ± 0.5	10	35.4 ± 7.9	10	12.9 ± 9.2
	High	3	9	26.5 ± 19.4	9	100.7 ± 23.9	9	13.0 ± 7.4
			37	**8.7 ± 4.8**	**37**	**87.7 ± 15.7**	**37**	**11.5 ± 3.0**

There was no significant linear correlation between total available soil mineral N and NUE (ORPHEE $p = 0.406$, IDENT-SSM $p = 0.452$), or between PO_4 and PUE (ORPHEE $p = 0.394$, IDENT-SSM $p = 0.779$). Birch foliage showed a higher N concentration than pine ($p < 0.001$), with values ranging on average between 22.89 ± 0.82 mg N g^{-1} in Be at ORPHEE and 24.83 ± 0.57 mg N g^{-1} in Ba at IDENT, compared with 9.07 ± 0.26 mg N g^{-1} in Pp and 19.33 ± 0.48 mg N g^{-1} in Ps, respectively (Table S4.2). Additionally, birch

was the species that tended to be affected by the H_2O treatment at both sites (ORPHEE $p = 0.095$, IDENT $p = 0.053$), with leaf concentration levels decreasing to 20.25 ± 0.62 mg N g^{-1} in Be at ORPHEE and 21.79 ± 0.41 mg N g^{-1} in Ba at IDENT in the high H_2O treatments (Table S4.2).

4.3.2 Effect of species richness and water availability on NutUE

At IDENT-SSM, SR had a significant ($p < 0.001$) positive effect on ANPP, litterfall mass, litter N, P, and Mg concentrations, but no effect on NutUE of these three elements (Table 4.1, Fig. 4.5, Fig. S4.3). Post-hoc tests revealed that the 2-species mixtures had significantly higher litter N ($p < 0.001$) and P ($p = 0.029$) concentrations than the monocultures. The four-species mixtures also had significantly ($p = 0.049$) higher litter P concentrations than the monocultures. Litter concentration and NutUE of other macronutrients (K, Ca, and Mn) were independent of SR (Table 4.1, Fig. S4.3). Water availability had no effect on ANPP, litterfall mass, litter nutrient concentrations, or NutUE except for Mg and Mn. Litter Mg was lower in the low H_2O treatment, while litter Mn concentration was higher in the low H_2O treatment (Table 4.1, Fig. S4.3). Nutrient use efficiency of Mg was significantly higher in the low H_2O treatment. Aboveground productivity, litterfall mass and nutrient concentrations, and NutUE were independent of SR × H_2O effects.

At the ORPHEE site, SR tended to positively affect ANPP ($p = 0.064$), but had no effect on litterfall mass (Table 4.1 & Fig. S4.4). There was a significant, negative effect of SR on the litter concentrations of P ($p = 0.034$) and K ($p = 0.038$), but SR did not affect litter N, Ca, Mg or Mn concentrations (Table 4.1, Fig. S4.4, Fig. S4.5), However, the combined influence of SR on ANPP, litterfall mass, and litter nutrient concentrations resulted in significant ($p \leq 0.042$), positive effects of SR on NUE, PUE, KUE, MgUE, and MnUE (Table 4.1). Both NUE and PUE were higher in the 2- and 3-species mixtures than the monocultures (Fig. S4.4). Similar positive effects of SR were observed for KUE, MgUE and MnUE (Table 4.1, Fig. S4.5). Water availability did not affect ANPP, litterfall mass, or NutUE, but litter Ca concentrations were significantly higher in the high H_2O treatment (Table 4.1, Fig. S4.5). The SR × H_2O effect was significant only for CaUE, which increased with SR in the low H_2O treatment, but remained constant and low in the high H_2O treatment (Table 4.1, Fig. S4.5).

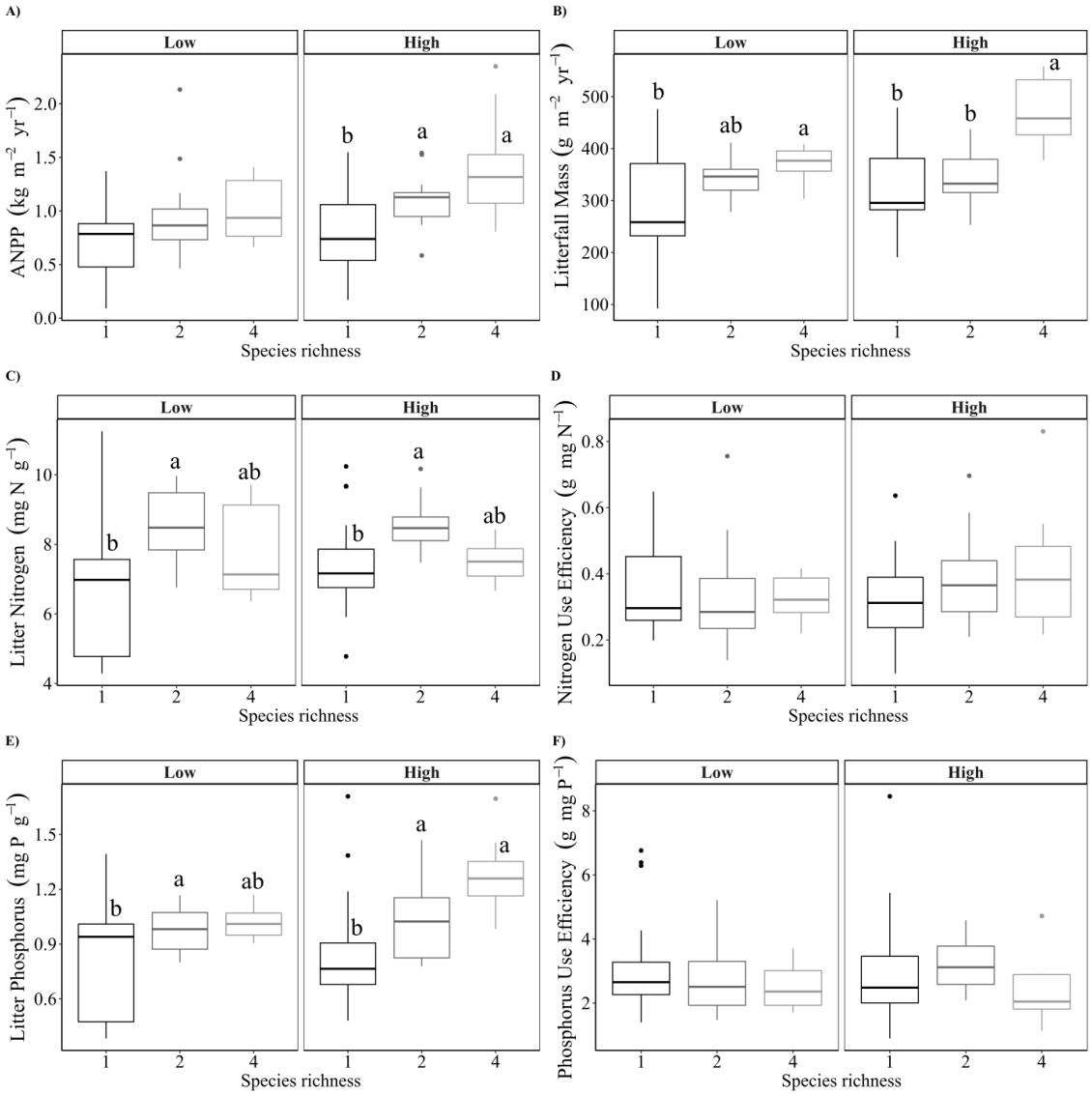

Figure 4.5: Box and stem plots showing the effect of tree species richness (1, 2 or 4 species) and water availability treatment (low or high H_2O) on A) aboveground net primary productivity (ANPP), B) litterfall mass, C) litter nitrogen (N) concentration, D) nitrogen use efficiency (NUE), E) litter phosphorus (P) concentration, and F) phosphorus use efficiency (PUE) at the IDENT-SSM site. Corresponding graphs at the ORPHEE site are in Fig. S4.4. Pairs of panels lacking letters indicate no overall effect (Table 4.1). SR means with different lowercase letters within an H_2O treatment are significantly different ($p < 0.05$, Tukey's HSD test).

4.3.3 Species identity and water availability effects on nutrient relations of mixed species communities

At both sites, species identity effects on nutrient relations in mixtures were driven largely by the tree species exhibiting the greatest aboveground growth in monocultures. There was a significant effect of species identity on ANPP and litterfall mass ($p < 0.001$) at ORPHEE: Be was characterized by significantly lower ANPP than Pp or Be-Pp (Table

S4.3 & Fig. 4.6AB). Water availability also had a significant, positive effect on ANPP and litterfall mass, that varied with species identity (Table S4.3 & Fig. 4.6AB). Species identity had a significant effect on litter nutrient concentrations of all elements; concentrations were significantly higher in Be compared with Pp and Be-Pp plots (Table S4.3, Fig. 4.6, Fig. S4.6). Lower litter nutrient concentrations in Be-Pp were due to the much larger contribution of relatively nutrient-poor, litterfall mass of Pp in this mixture (Fig. 4.6B). Water treatment had a significant effect only on litter Ca and Mn concentrations, being larger in the high H_2O treatment. Due to differences in productivity and litter nutrient concentrations of the two species when grown alone or in mixture, we observed a significant species identity effect on NutUE of all elements examined (Table S4.3, Fig. 4.6, Fig. S4.6). Nutrient use efficiency of Be was always lower than Be-Pp, with Be-Pp NutUE being higher than the two monocultures for some elements and H_2O treatments (Table S4.3, Fig. 4.6, Fig. S4.6). Overall, there was no effect of species identity or H_2O treatment on NutRE except for K, that had lower values in Be but differed with H_2O treatment (Table S4.3, Fig. 4.8).

In contrast, species identity and H_2O treatment effects on nutrient relations of birch-pine mixtures at IDENT-SSM were largely determined by the dominance of Ba over Ps. There was a significant ($p < 0.001$) effect of species identity on ANPP and litterfall mass, with Ba having significantly lower values than the Ps and Ba-Ps plots (Table S4.3, Fig. 4.7AB). Species identity had a significant ($p < 0.001$) effect on all litter nutrient concentrations except Mn (Table S4.3). Litter nutrient concentrations were higher in Ba and Ba-Ps than Pp, with litter Mg concentrations being significantly higher in the high H_2O treatment (Fig. 4.7 & Fig. S4.7). There was also a significant species identity × H_2O effect, such that the species identity effect on litter N and P concentrations were diminished in the high H_2O treatment, but litter Ca concentration was accentuated by the high H_2O treatment (Table S4.3, Fig. 4.7, Fig. S4.7). Litter NutRE differed with species identity for P, Ca, Mg, and Mn, with the species identity effects on CaRE and MgRE being diminished in the high H_2O treatment. The Ps plots exhibited lower NRE than the Ba and Ba-Ps communities, only in the high H_2O treatment due to a significant species identity × H_2O interaction (Table S4.3, Fig. 4.8). Species identity had a significant effect on NutUE of all elements but Mn, and were significantly higher in the Ps than Ba plots in the high (CaUE) or both H_2O treatments (Table S4.3, Fig. 4.8, Fig. S4.7). Nutrient use efficiency of Ba-Ps was lower than Ps only for MgUE and CaUE (Fig. 4.7, Fig. S4.7).

The effect of mixing on productivity and nutrient relations of birch and pine under high and low water conditions at both sites is summarized in Figure 4.9. At ORPHEE, Pp exhibited significantly greater ANPP under both H_2O treatments, and higher litterfall mass in the high H_2O treatment when grown in mixture with Be. Mixing also significantly increased both NUE and PUE of Pp when under water limited conditions. By comparison,

Be derived no apparent, significant benefit from being grown in mixture with Pp in either H_2O treatment. At IDENT, Bp exhibited significantly higher productivity (ANPP, litterfall mass) under low and high water availability when grown in mixture with Ps, while Ps exhibited the opposite effect of mixing with Ba.

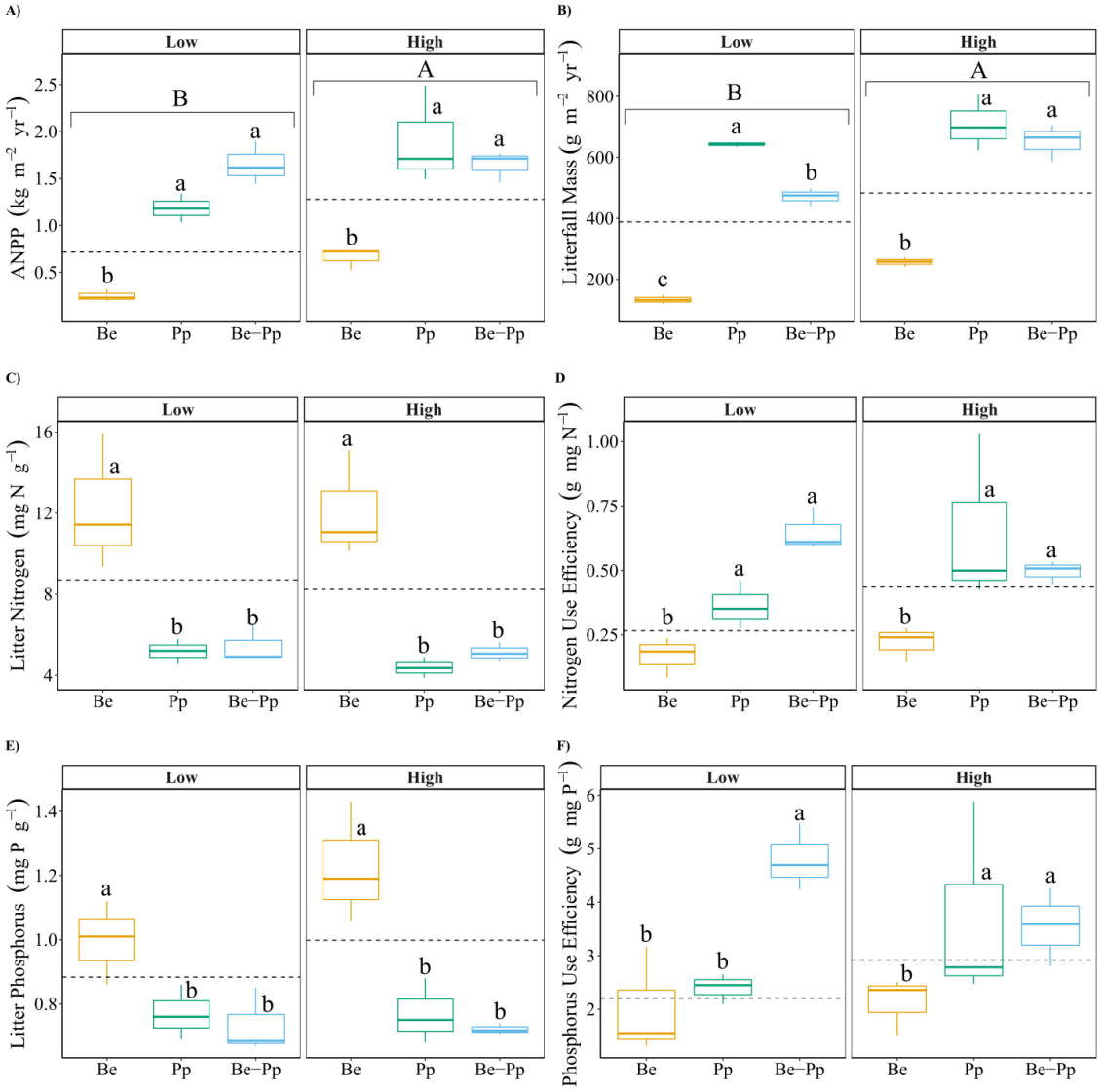

Figure 4.6: Box and stem plots showing the effects of species identity and water availability treatment (low or high H_2O) on A) aboveground net primary productivity (ANPP), B) litterfall mass C) litter nitrogen (N) concentration, D) nitrogen use efficiency (NUE), E) litter phosphorus (P) concentration, and F) phosphorus use efficiency (PUE) for *Betula pendula* (Be), *Pinus pinaster* (Pp), and Be-Pp mixtures at the ORPHEE site. The horizontal dashed line represents the expected value for a 50% Be-Pp mixture value. Pairs of panels lacking letters indicate no overall effect (Table 4.1). SR means with different lowercase letters within an H_2O treatment are significantly different ($p < 0.05$, Tukey's HSD test). Significant overall H_2O treatments are indicated by uppercase letters ($p < 0.05$).

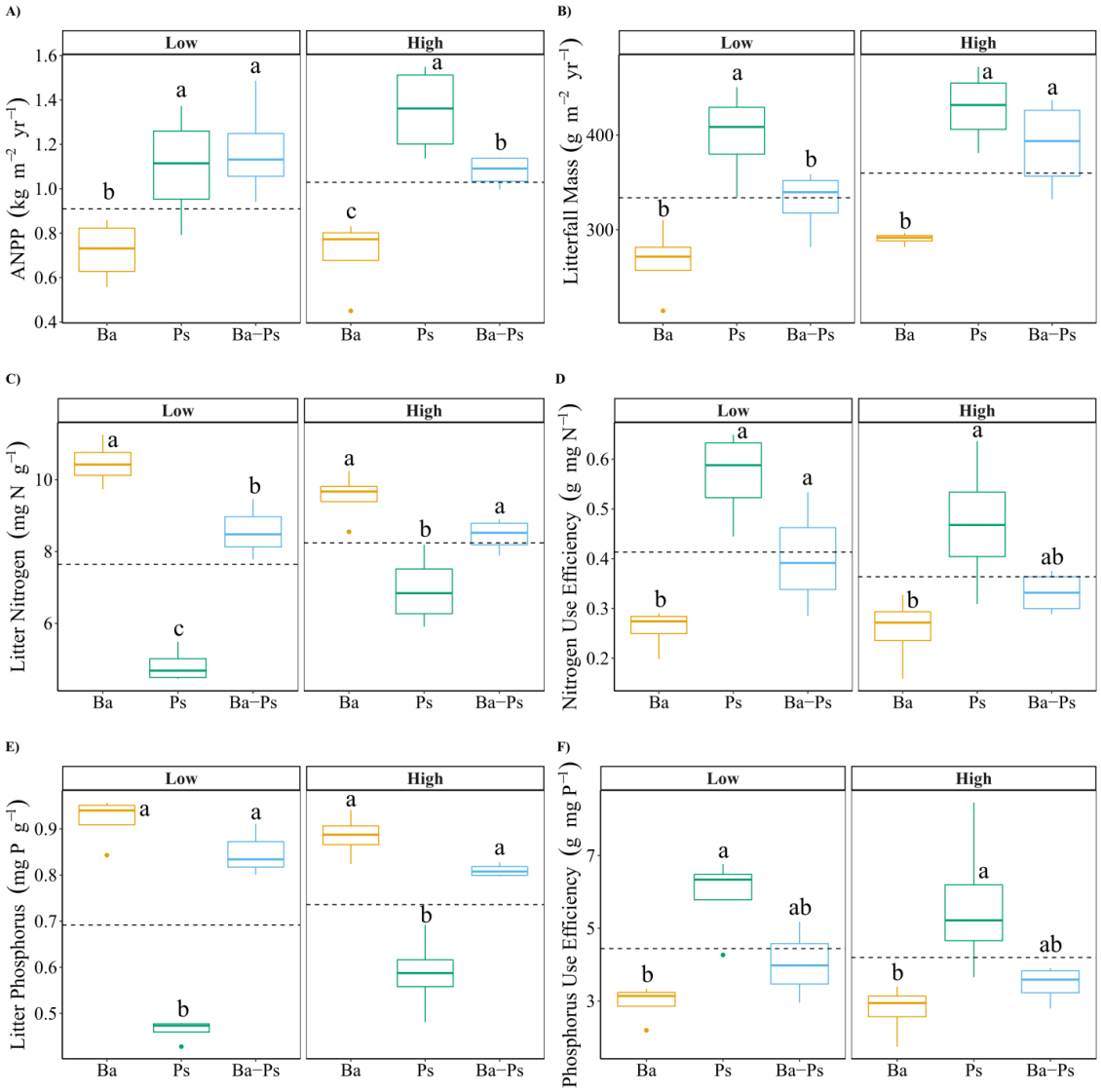

Figure 4.7: Box and stem plots showing the effects of species identity and water availability treatment (low or high H_2O) on A) aboveground net primary productivity (ANPP), B) litterfall mass C) litter nitrogen (N) concentration, D) nitrogen use efficiency (NUE), E) litter phosphorus (P) concentration, and F) phosphorus use efficiency (PUE) for *Betula papyrifera* (Ba), *Pinus strobus* (Ps), and Ba-Ps mixtures at the IDENT-SSM site. The horizontal dashed line represents the expected value for a 50% Ba-Ps mixture value. Pairs of panels lacking letters indicate no overall effect (Table 4.1). SR means with different lowercase letters within an H_2O treatment are significantly different ($p < 0.05$, Tukey's HSD test).

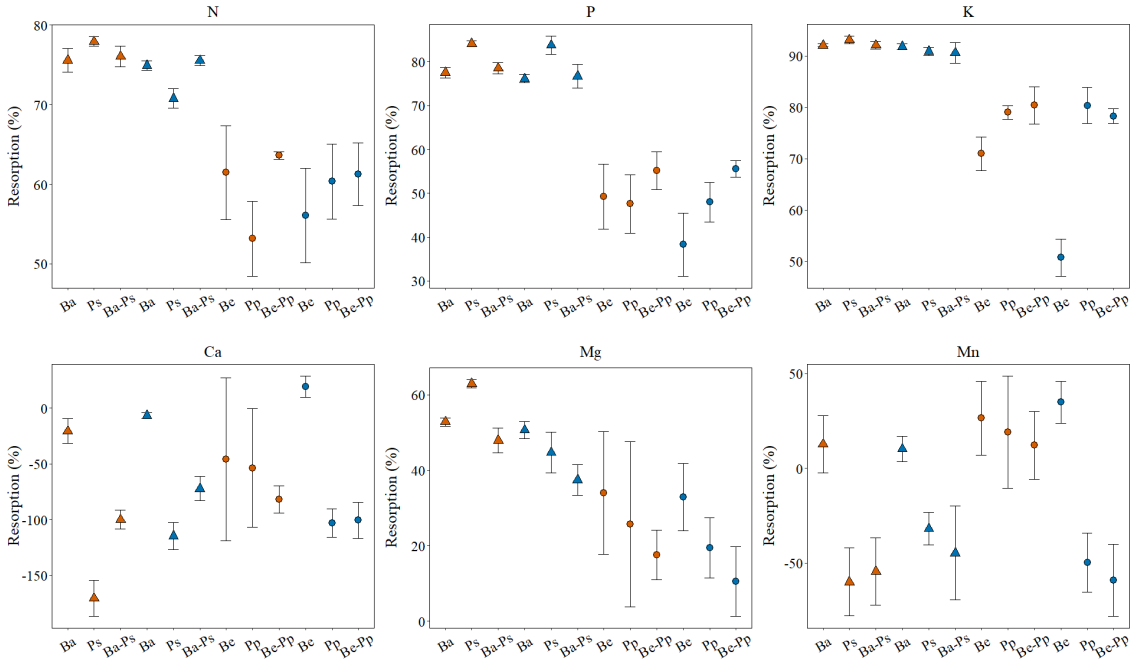

Figure 4.8: Mean (± standard error) nutrient resorption efficiency (%) for N, P, K, Ca, Mg, Mn for birch, pine, and birch-pine mixtures at the IDENT-SSM (triangles) and ORPHEE (circles) sites, in the low (red symbols) and high (blue symbols) water availability treatments. Results for IDENT-SSM appear to the left of the vertical dashed line in each figure. Species abbreviations: *Betula papyrifera* (Ba), *Pinus strobus* (Ps), *Betula pendula* (Be), and *Pinus pinaster* (Pp).

	IDENT-SSM				ORPHEE			
	Birch (*Ba*)		Pine (*Ps*)		Birch (*Be*)		Pine (*Pp*)	
	Low	High	Low	High	Low	High	Low	High
ANPP	↗ *	↗ **	↘	↘ **	↗	↘	↗ **	↗ *
Litterfall mass	↗ ***	↗ **	↘ **	↘ ***	↗	↘	↗	↗ *
Litter N	↘ *	=	↗	=	↘	=	↘	=
NRE	=	=	=	↗ **	↗	=	↗	=
NUE	↗	=	↗	↗	↗	↘	↗ *	=
Litter P	↗	↗	↗	=	↘	↘	↘	↘
PRE	=	=	=	=	↗	↗	↗	↗
PUE	=	=	↗	=	↗	=	↗ *	=

Figure 4.9: Significant effects and trends on productivity and nutrient relations for birch (Ba, Be) and pine (Ps, Pp) under low and high water availability treatment that resulted from growth in mixture at the ORPHEE (Be, Pp) and IDENT-SSM (Ba, Ps) sites. Arrows represent positive or negative effects on performance of birch and pine in mixture, compared with their respective performance in monocultures for aboveground net primary productivity (ANPP), litterfall mass, litter N concentration, nitrogen resorption efficiency (NRE), nitrogen use efficiency (NUE), litter P concentration, phosphorus resorption efficiency (PRE), and phosphorus use efficiency (PUE). Neutral effects are represented by =. Small arrows represent trends, and large arrows with asterisks represent significant differences from Welch two sample t-tests between the expected and observed values for the mixed species plot ($p < 0.05$*, $p < 0.01$**, $p < 0.001$***). Corresponding values can be found in Table S4.4.

4.4 Discussion

4.4.1 Species richness increases productivity, but not NutUE

Examining the different components of the NutUE equation, we noted an overall positive effect of SR on ANPP and litterfall mass at both sites. The productivity of birch and larch tended to be stable or increased in mixtures at IDENT-SSM, but that of pine, maple and oak decreased in mixtures (Belluau et al. 2021). At ORPHEE, the positive effect of SR on ANPP was due to the dominance of pine over birch, as previously measured 7 years after plantation (Morin et al. 2020). Species combinations that favor light interception may drive positive diversity-productivity relationships in forests (Jucker et al. 2014b; Morin et al. 2011), due to complementarity among species with different shade tolerance, growth rate and crown architecture (Cordonnier et al. 2018a; Jucker et al. 2015; Kelty 2006). At IDENT-SSM, birch was dominant (in all mixtures where it is present) and composed between 50 and 75% of the litterfall mass in the 4- and 2-species mixtures, respectively. This resource acquisitive species was able to grow more rapidly and dominated the small plot canopy space at IDENT-SSM after 6 years of growth (Belluau et al. 2021). At ORPHEE, birch also performed better in height growth than pine in the first four years of growth (Morin et al. 2020), but then pine growth overtook that of birch. Pine then dominated the productivity and canopy packing in mixtures (Martin-Blangy et al. submitted), with a proportion of around 90% of the litterfall mass.

SR strongly influenced plot-level litterfall nutrient dynamics, but in opposite directions at the two sites. At IDENT-SSM, litter N and P concentrations were significantly higher in the 2-species mixtures compared with the monocultures under both levels of water availability (Fig. 4.5), without any changes in nutrient resorption efficiency (NutRE). At ORPHEE, the effect of SR on litter nutrient concentrations was negative, both due to the strong contribution of nutrient-poor pine litter to the mixture litter mass (Fig. S4.4), and to an increase in NutRE with mixing. The differences in plot density and age could explain these differing leaf nutrient dynamics. In the dense IDENT-SSM plots, litterfall mass was dominated by nutrient-rich species, whose decomposition could have led to an increase in nutrient availability (Gartner and Cardon 2004; Hättenschwiler et al. 2005), subsequently taken up by the trees in the mixture, and increasing overall leaf nutrient concentrations. These young plots were less inclined to acclimate their already-high NutRE values to changes in plot-level nutrient concentrations, as a high NutRE is useful in early years of growth (Colin-Belgrand et al. 1996). At ORPHEE, the strong contribution of nutrient-poor pine litter to the litterfall mass decreased the plot-level litter nutrient concentrations. This was accompanied by an increase in NutRE in both birch and pine in the mixture (Fig. 4.8, Fig. 4.9), which may be due to higher nutrient demand in the mixtures where tree growth

was enhanced. However, this pattern was not observed in the nutrients extracted from the ion-exchange resins. We found no link between soil nutrient availability and either nitrogen use efficiency (NUE) or phosphorus use efficiency (PUE; Fig. S4.2). These results are in line with those of Knops et al. 1997, who found no relationship between NutUE and site fertility. However, the evaluation of soil nutrient availability *via* the nutrients extracted from the resins is somewhat limited, as nutrients made available by forest floor decomposition may have been immediately taken up by roots in the topsoil or even directly from the forest floor layer. While the resins were placed at 8 cm depth, roots in temperate forests have been found to be highly dense in the forest floor and first 5 cm of mineral soil (Altinalmazis-Kondylis et al. 2020; Archambault et al. 2019; Bakker et al. 2006).

The combination of increased ANPP and litterfall nutrient concentration with SR led to no effect of SR on NutUE at IDENT-SSM. At ORPHEE, the increase in productivity (ANPP and litterfall mass) in the mixtures accompanied by a decrease in litterfall nutrient concentration led to an increase in plot-level NutUE with SR. The trends leading to the contradiction or support for first hypothesis (H_1) at each site were driven by species composition, and varied due to the different plot age and densities. Our results follow others noted in tropical ecosystems, in which the identity of species within a mixture determined ecosystem NutUE rather than species richness more generally (Hiremath and Ewel 2001; Tang et al. 2010). More specifically, the interaction between birch, a resource-acquisitive species, and pine, a resource-conservative species, in the different planting contexts (i.e. plot age and density), was more important than SR itself at each site.

4.4.2 Minimal effect of water availability on NutUE

Contrary to our second hypothesis (H_2), we found no effect of water availability on NutUE at IDENT-SSM or ORPHEE. The contrasting low (*via* rain exclusion) vs high (*via* irrigation) water availability (H_2O) treatments at IDENT-SSM led to only a marginal increase in litterfall mass with high H_2O (Fig. 4.5). Generally, increasing water availability has been found to increase tree productivity (Mayor and Rodà 1994; Waterworth et al. 2007). At ORPHEE, however, high H_2O tended to increase ANPP and litterfall mass, but mainly in the monocultures (Fig. S4.4). Because SR increased ANPP, the lack of an overall effect of water availability at the plot-level in these mixtures indicates that water was not the main limiting growth factor in these plots. The lack of strong effects of water availability at our sites suggests that nutrients were more limiting than water (Albaugh et al. 2016). This limitation was likely higher in the poor sandy soils of the ORPHEE region, where a widespread and severe limitation in phosphorus has been previously found (Trichet et al. 2009). This was consistent with the fact that green leaf nutrient concentrations were on the cusp of adequate N and P concentrations for these species (CSIRO 1997;

van den Burg 1985). In contrast, species at IDENT-SSM exhibited green leaf nutrient concentrations within their optimum nutrition ranges (Table S4.2), thus confirming that the nutrient limitation was stronger at ORPHEE.

There was no effect of water availability on litter N or P concentrations at both sites (Table 4.1, Fig. 4.5 & Fig. S4.4), in contrast to the results of a dry-season irrigation experiment in a tropical forest regrowth (Vasconcelos et al. 2008). This confirms that the plots were more limited by nutrients than by water, as discussed above (Trichet et al. 2008), although light interception of the canopy also may play a role in these young and dense stands (Martin-Blangy et al. submitted; Williams et al. 2017). Nonetheless, we found significant positive effects of high H_2O on litter Mg concentrations at IDENT-SSM (Fig. S4.3), and litter Ca and Mn concentrations at ORPHEE (Fig. S4.5, Table 4.1). The Ca and Mg elements can be passively accumulated in the tree transpiration stream after active channel uptake, and then accumulate in the xylem and in leaves (Achat et al. 2018; Augusto et al. 2011) because they are poorly remobilized through phloem (Marschner 2012). The transporters for Mn are relatively aspecific, which may make it possible to use leaf [Mn] as a proxy for carboxylates in the rhizosphere (Lambers et al. 2015). The results on Mn may help reveal the strategy for plant P acquisition, as mycorrhizal fungi intercept Mn whereas carboxolates mobilize Mn (Pang et al. 2018). Improved water supply should boost evapotranspiration, leading to an increase in these cations recovered in the leaf litter (Achat et al. 2018). Consequently, with this increase in litter cations, we noted a negative effect of high H_2O on MgUE at IDENT-SSM (Fig. S4.3), and at ORPHEE a slight negative effect of high H_2O on MnUE and a similar trend on CaUE (Fig. S4.5).

With regards to our third hypothesis (H_3), we did not find an interactive effect between SR and water availability on NutUE values. At IDENT-SSM, we did note positive trends of SR on ANPP and litterfall mass under high H_2O, as previously observed at this site (Belluau et al. 2021). However, this interactive effect was not reflected in the NutUE values. At ORPHEE, when we tested the specific effect of species composition, instead of species richness, significant interactive effects on productivity emerged. High H_2O increased the monoculture ANPP, but had little effect on mixed plot-level ANPP, probably because the canopy in ORPHEE's mixtures was already packed, and trees in the mixtures were not as advantaged with an increase in water availability as their respective trees in the monocultures (Martin-Blangy et al. submitted). The effect of species composition within a mixture is thus of interest to further explore mixture dynamics.

4.4.3 Birch-pine dynamics drive species interactions at both levels of water availability

In order to better interpret the interactions between species identity and water availability, we focused on birch and pine, two genera present at both experimental sites. Specifically, we focused on the pure birch (Ba at IDENT-SSM, Be at ORPHEE), pure pine (Ps at IDENT-SSM, Pp at ORPHEE), and the 2-species mixtures (Ba-Ps & Be-Pp). Interestingly, pine had higher NutUE than birch at both sites. This observation agrees with Vitousek 1982, who found that the efficiency of litterfall production per N and P was higher in temperate coniferous compared to deciduous forests. Coniferous species are resource conservative, having evolved in nutrient poor and xeric ecosystems, and are consequently characterized by higher NutUE and lower litter nutrient quality than deciduous species (Augusto et al. 2014; Berendse and Scheffer 2009; Chapin et al. 1993; Pastor et al. 1984). Combining these strategies in plantations is thus of interest to explore the effect of mixing species on plot-level NutUE. Trends in nutrient dynamics in the birch-pine mixtures at each site were based on the combination of differing resource acquisition strategies, tree density and stand age.

In the first years of growth, birch grew more rapidly at both sites, and was able to gain advantage over pine and dominate the available canopy space in the dense young plots of IDENT-SSM, leading to productivity overyielding with mixing (Fig. 4.7AB). With an increase in growth there was an increase in leaf litterfall nutrient concentrations, due to the synergistic non-additive presence of nutrient-rich birch litter. This led to an additive effect of mixing birch and pine on NUE and PUE (Fig. 4.7DF). In the older and less dense plots at ORPHEE, pine caught up and overtook the birch trees (Morin et al. 2020), and had more canopy space to grow than pines in the monocultures, leading to overyielding at the site (Fig.4.6AB). The strong presence of pine in the litterfall production decreased plot-level litterfall nutrient concentrations, thereby increasing NUE and PUE in the mixed birch-pine plots at ORPHEE (Fig. 4.6DF). Thus, mixing a resource acquisitive species such as birch and a resource conservative species such as pine in dense plots will initially favor the former, which will fill the available canopy space (Williams et al. 2017) and may improve both plot-level productivity and leaf nutrient concentrations in the first few years of growth. However, growing these two species types in lower density plots allows the resource conservative species to 'catch up' with age, and may thus improve plot-level NutUE.

Mixing was a strong driver for NutUE, and after four years of the irrigation treatment, plot-level NUE and PUE increased more with mixing in the low water availability treatment at ORPHEE (Fig. 4.9). Indeed, mixing birch and pine, rather than SR itself, had a more positive effect when water resources were low. Stronger biodiversity-ecosystem

functioning relationships have been previously found in drier areas with more functionally diverse tree species (Ratcliffe et al. 2017), where the growth rates are lower (del Rio et al. 2014). We did not however find this same trend at IDENT-SSM, where the effect of mixing birch and pine was additive under both water availability treatment levels (Fig. 4.9). Despite a small interactive effect at one site, species composition of the mixtures (species identity) appeared to be more important than water availability for plot-level NutUE.

4.5 Conclusions

Our study is the first to test the interactive effect of stand composition (i.e. species richness and species identity) and water availability on nutrient use efficiency (NutUE). By using two rare experimental sites that manipulated both of these factors, we demonstrated that species' dominance drove productivity responses, litterfall nutrient dynamics, and ultimately NutUE in mixed species plantations. More specifically, the interactions between birch and pine (a deciduous broadleaf and an evergreen conifer species) drove mixing effects, but differently at each site probably due to differences in plot age and density. Younger and denser stands may favor the production of nutrient-rich birch, and may not improve stand-level NutUE, which may have repercussions for future stand-level productivity. In older stands, the production of nutrient-poor pine is favored, leading to an increase of stand-level NutUE at the detriment of birch productivity. This was true for plots under both low and high water availability treatments. Overall, we conclude that species composition is a stronger driver for NutUE than water availability, and that mixing certain tree species can be a viable way to improve stand-level NutUE in forests provided that the complementarity of species is correctly assessed.

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4.6 Supplementary information

Table S4.1: Descriptions of both experimental sites in 2019.

	IDENT-SSM, Canada	ORPHEE, France
Tree plantation year	2013	2008
Mean annual precipitation (mm)	899	870
Mean annual temperature (ºC)	4.7	12.5
Soil texture	sandy loam ∼loamy fine sand	sandy
Soil pH [H_2O]	5.4	4.5
Plot size	49 trees in 10.24 m²	100 trees in 400 m²
Blocks (water availability treatment)	4 rain exclusion (low H2O), 4 irrigation (high H_2O)	4 control (low H_2O), 4 irrigation (high H_2O)
Number of species	1, 2, 4	1, 2, 3
Species composition	*Betula papyrifera* (Ba), *Pinus strobus* (Ps), *Acer saccharum* (As), *Larix laricina* (Ll), *Quercus rubra* (Qu), Ba-Ps, Ba-As, Ba-Ll, Qu-Ll, Qu-As-Ll-Ps, Ba-As-Ll-Ps	*Betula pendula* (Be), *Pinus pinaster* (Pp), *Quercus robur L.* (Qo), Be-Pp, Be-Pp-Qo

Table S4.2: Green leaf nutrient concentrations for each species within each irrigation treatment, at each site. Data are mean ± standard error of the mean.

Site	Species	H_2O	n	Green leaf N (mg N g^{-1})	Green leaf P (mg P g^{-1})
ORPHEE	*Betula pendula* (Be)	Low	9	22.89 ± 0.82	1.59 ± 0.06
	Betula pendula (Be)	High	9	20.25 ± 0.62	1.45 ± 0.04
	Pinus pinaster (Pp)	Low	9	9.07 ± 0.26	1.23 ± 0.04
	Pinus pinaster (Pp)	High	9	8.87 ± 0.48	1.14 ± 0.05
IDENT-SSM	*Acer saccharum* (As)	Low	16	15.66 ± 0.94	1.39 ± 0.11
	Acer saccharum (As)	High	16	17.6 ± 0.92	1.97 ± 0.11
	Betula papyrifera (Ba)	Low	20	24.83 ± 0.57	2.54 ± 0.07
	Betula papyrifera (Ba)	High	20	21.79 ± 0.41	2.13 ± 0.11
	Larix laricina (Ll)	Low	20	21.75 ± 0.43	2.5 ± 0.08
	Larix laricina (Ll)	High	20	22.8 ± 0.33	2.29 ± 0.17
	Pinus strobus (Ps)	Low	16	19.33 ± 0.48	2.53 ± 0.07
	Pinus strobus (Ps)	High	16	19.77 ± 0.45	2.65 ± 0.2
	Quercus rubra (Qu)	Low	12	23.02 ± 0.74	1.41 ± 0.04
	Quercus rubra (Qu)	High	12	23.34 ± 0.9	1.33 ± 0.11

Table S4.3: Results of mixed model analysis of species identity, water availability treatment (H_2O) and their interaction on productivity and nutrient relations at the IDENT-SSM and ORPHEE sites. F-values and p-values for fixed effects are presented. Significant ($p < 0.05$) effects are noted in bold font. Abbreviations as defined earlier.

		Species identity (Plot)		H_2O		Plot x H_2O	
		F-value	p-value	F-value	p-value	F-value	p-value
IDENT-SSM	ANPP	20.044	**<0.001**	0.308	0.618	2.227	0.150
	Litterfall mass	24.297	**<0.001**	5.341	0.104	0.484	0.628
	Litter N	153.646	**<0.001**	4.948	0.113	27.812	**<0.001**
	Litter P	124.521	**<0.001**	2.445	0.216	8.496	**0.006**
	Litter K	26.123	**<0.001**	0.009	0.930	3.310	0.075
	Litter Ca	56.910	**<0.001**	0.062	0.819	12.782	**0.001**
	Litter Mg	519.111	**<0.001**	61.888	**0.004**	2.465	0.130
	Litter Mn	0.223	0.804	7.706	0.069	0.482	0.630
	NUE	16.433	**<0.001**	1.776	0.275	0.298	0.748
	PUE	15.352	**0.001**	0.859	0.422	0.025	0.976
	KUE	8.043	**0.007**	0.088	0.786	0.121	0.887
	CaUE	8.255	**0.006**	0.192	0.691	4.724	**0.033**
	MgUE	60.139	**<0.001**	45.341	**0.007**	2.221	0.155
	MnUE	0.915	0.429	1.036	0.384	0.299	0.748
	NRE	1.811	0.209	8.443	0.062	9.297	**0.004**
	PRE	15.211	**0.001**	0.857	0.423	0.150	0.862
	KRE	0.285	0.758	3.162	0.173	0.691	0.523
	CaRE	70.987	**<0.001**	12.653	**0.012**	1.912	0.198
	MgRE	6.170	**0.018**	15.439	**0.008**	3.454	0.072
	MnRE	10.402	**0.004**	0.923	0.374	0.559	0.588
ORPHEE	ANPP	156.635	**<0.001**	21.399	**0.044**	15.058	**0.002**
	Litterfall mass	572.245	**<0.001**	113.659	**0.009**	23.893	**<0.001**
	Litter N	53.373	**<0.001**	0.499	0.553	0.492	0.629
	Litter P	30.756	**<0.001**	2.123	0.282	2.173	0.176
	Litter K	10.409	**0.006**	2.962	0.227	0.062	0.940
	Litter Ca	19.532	**0.001**	40.807	**0.024**	1.104	0.377
	Litter Mg	61.823	**<0.001**	1.716	0.320	0.671	0.538
	Litter Mn	21.012	**0.001**	24.451	**0.039**	0.258	0.779
	NUE	26.137	**<0.001**	1.977	0.295	3.039	0.104
	PUE	17.392	**0.001**	0.282	0.649	3.779	0.070
	KUE	23.936	**<0.001**	0.442	0.574	4.383	0.052
	CaUE	6.047	**0.025**	2.759	0.239	5.752	**0.028**
	MgUE	37.650	**<0.001**	0.255	0.664	5.184	**0.036**
	MnUE	22.853	**<0.001**	11.870	0.075	1.865	0.216
	NRE	0.762	0.498	0.002	0.968	0.991	0.413
	PRE	2.697	0.127	0.663	0.501	0.828	0.471
	KRE	31.654	**<0.001**	10.015	0.087	9.188	**0.008**
	CaRE	2.062	0.198	0.000	0.995	1.107	0.382
	MgRE	1.113	0.381	0.235	0.676	0.029	0.971
	MnRE	4.580	0.053	7.850	0.107	2.438	0.157

Table S4.4: Mean (± 1 standard deviation) of litter mass, aboveground net primary productivity (ANPP), litter nitrogen (N) concentration, N resorption efficiency (NRE), N use efficiency (NUE), litter phosphorus (P) concentration, P resorption efficiency (PRE) and P use efficiency (PUE) for *Betula pendula* (Be), *Betula papyrifera* (Ba), *Pinus pinaster* (Pp), and *Pinus strobus* (Ps) grown in monoculture or in mixture under high and low water availability (H_2O) at the ORPHEE and IDENT-SSM sites. Statistically significant differences are presented in Fig. 4.9.

Site	Sp.	H_2O	Mono vs. mixed	Litter mass g m^{-2} yr^{-1}	ANPP kg m^{-2} yr^{-1}	Litter N mg N g^{-1}	NRE %	NUE g mg^{-1} N	Litter P mg P g^{-1}	PRE %	PUE g mg^{-1} P
IDENT-SSM	Birch (Ba)	Low	mono-culture	133.47 ± 19.78	0.36 ± 0.07	10.46 ± 0.64	75.57 ± 2.95	0.26 ± 0.04	0.92 ± 0.05	77.52 ± 2.39	2.96 ± 0.52
	Birch (Ba)	Low	mixed	245.60 ± 17.86	0.76 ± 0.22	9.43 ± 0.57	76.89 ± 0.91	0.33 ± 0.10	0.95 ± 0.05	77.94 ± 2.87	3.27 ± 0.94
	Birch (Ba)	High	mono-culture	145.20 ± 3.19	0.35 ± 0.09	9.53 ± 0.71	74.9 ± 1.22	0.26 ± 0.07	0.88 ± 0.05	76.13 ± 1.94	2.76 ± 0.71
	Birch (Ba)	High	mixed	270.11 ± 31.26	0.67 ± 0.09	9.57 ± 0.35	74.31 ± 1.22	0.26 ± 0.01	0.93 ± 0.04	73.71 ± 7.09	2.67 ± 0.26
	Pine (Ps)	Low	mono-culture	200.27 ± 24.81	0.55 ± 0.13	4.83 ± 0.47	77.93 ± 1.18	0.57 ± 0.09	0.46 ± 0.02	84.12 ± 1.30	5.92 ± 1.12
	Pine (Ps)	Low	mixed	84.33 ± 47.47	0.41 ± 0.09	6.57 ± 1.54	74.22 ± 5.72	0.68 ± 0.22	0.61 ± 0.13	81.95 ± 3.59	7.22 ± 2.24
	Pine (Ps)	High	mono-culture	214.56 ± 19.95	0.68 ± 0.10	6.95 ± 1.01	70.76 ± 2.43	0.47 ± 0.14	0.59 ± 0.09	83.77 ± 4.09	5.64 ± 2.02
	Pine (Ps)	High	mixed	119.02 ± 20.40	0.41 ± 0.08	5.92 ± 0.48	78.31 ± 1.89	0.61 ± 0.18	0.55 ± 0.06	83.93 ± 2.34	6.46 ± 1.58
ORPHEE	Birch (Be)	Low	mono-culture	66.86 ± 7.60	0.13 ± 0.03	12.24 ± 3.34	61.46 ± 10.24	0.17 ± 0.08	1.00 ± 0.13	49.25 ± 12.79	2.01 ± 1.01
	Birch (Be)	Low	mixed	84.10 ± 47.64	0.22 ± 0.14	10.12 ± 1.26	66.79 ± 10.02	0.26 ± 0.10	0.87 ± 0.18	59.96 ± 11.48	3.02 ± 0.93
	Birch (Be)	High	mono-culture	128.71 ± 7.60	0.33 ± 0.06	12.1 ± 2.64	56.08 ± 10.31	0.22 ± 0.07	1.23 ± 0.19	38.33 ± 12.49	2.13 ± 0.53
	Birch (Be)	High	mixed	76.34 ± 31.87	0.18 ± 0.16	11.58 ± 2.22	58.72 ± 7.39	0.19 ± 0.07	0.95 ± 0.07	55.20 ± 3.42	2.3 ± 1.04
	Pine (Pp)	Low	mono-culture	321.36 ± 3.89	0.59 ± 0.08	5.18 ± 0.61	53.15 ± 8.12	0.36 ± 0.09	0.77 ± 0.09	47.58 ± 11.5	2.4 ± 0.28
	Pine (Pp)	Low	mixed	386.90 ± 60.58	1.44 ± 0.13	4.39 ± 0.25	63.22 ± 2.40	0.87 ± 0.20	0.71 ± 0.07	54.26 ± 6.95	5.36 ± 0.97
	Pine (Pp)	High	mono-culture	354.45 ± 46.04	0.95 ± 0.26	4.38 ± 0.51	60.35 ± 8.15	0.65 ± 0.33	0.77 ± 0.10	48.00 ± 7.77	3.71 ± 1.89

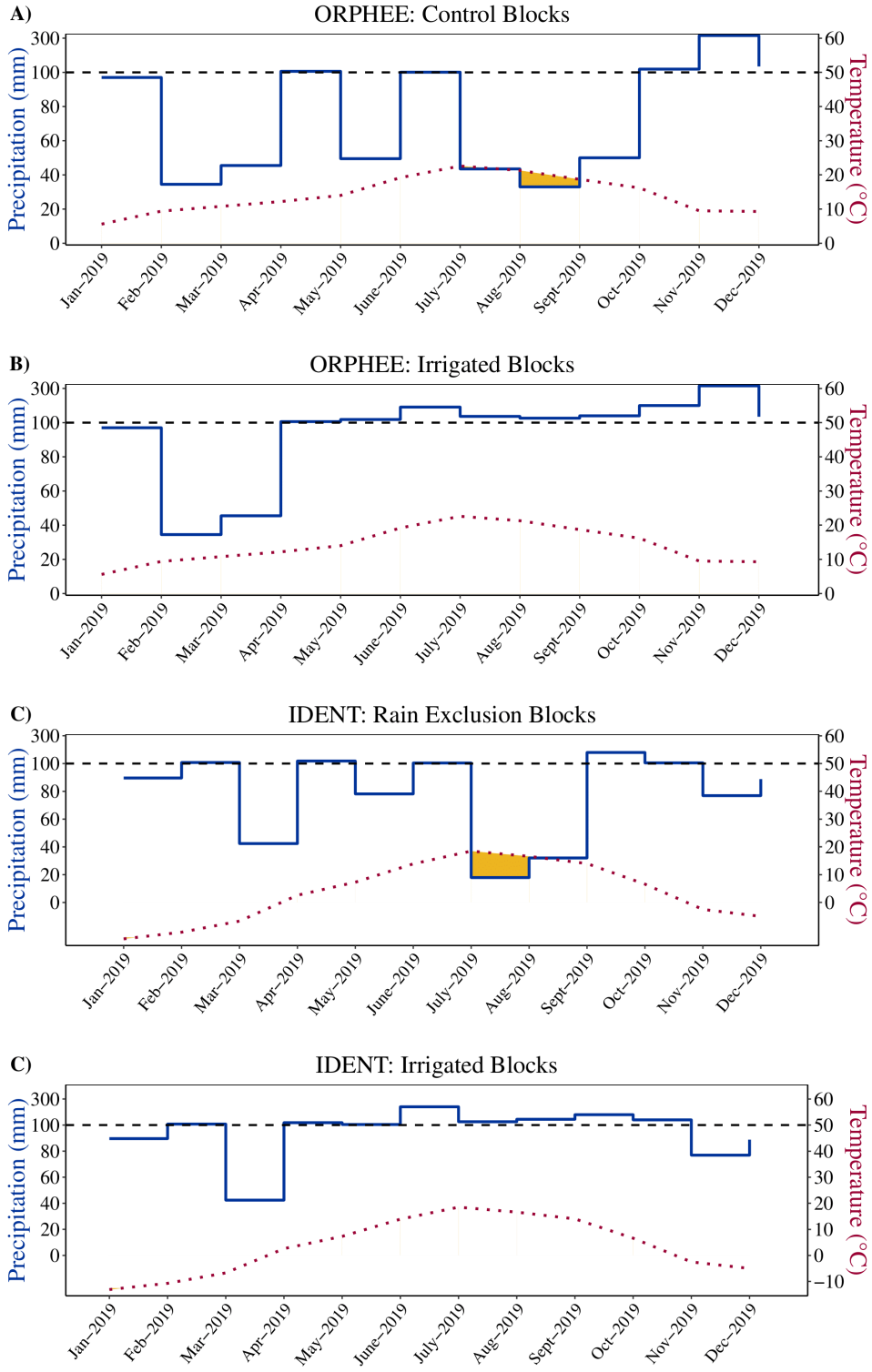

Figure S4.1: Ombrothermic diagram with total monthly precipitations (blue solid line) and average monthly temperature (red dotted line) during the study period for the high and low water treatments at both study sites. Data for ORPHEE were obtained from an INRAE weather station at Cestas Pierroton (44.742°N - 0.782°E), within 1 km of the experimental site. Data for IDENT-SSM were collected on site (May - October) and an Environment Canada Weather Station (November - April) within 10 km of the trial area. Temperature scale is set to 2mm/°C, and values above the 100mm black dashed horizontal line are scaled to 20mm/°C (Walter and Lieth, 1967). When temperature is 2x greater than precipitation, there is an arid period identified in yellow.

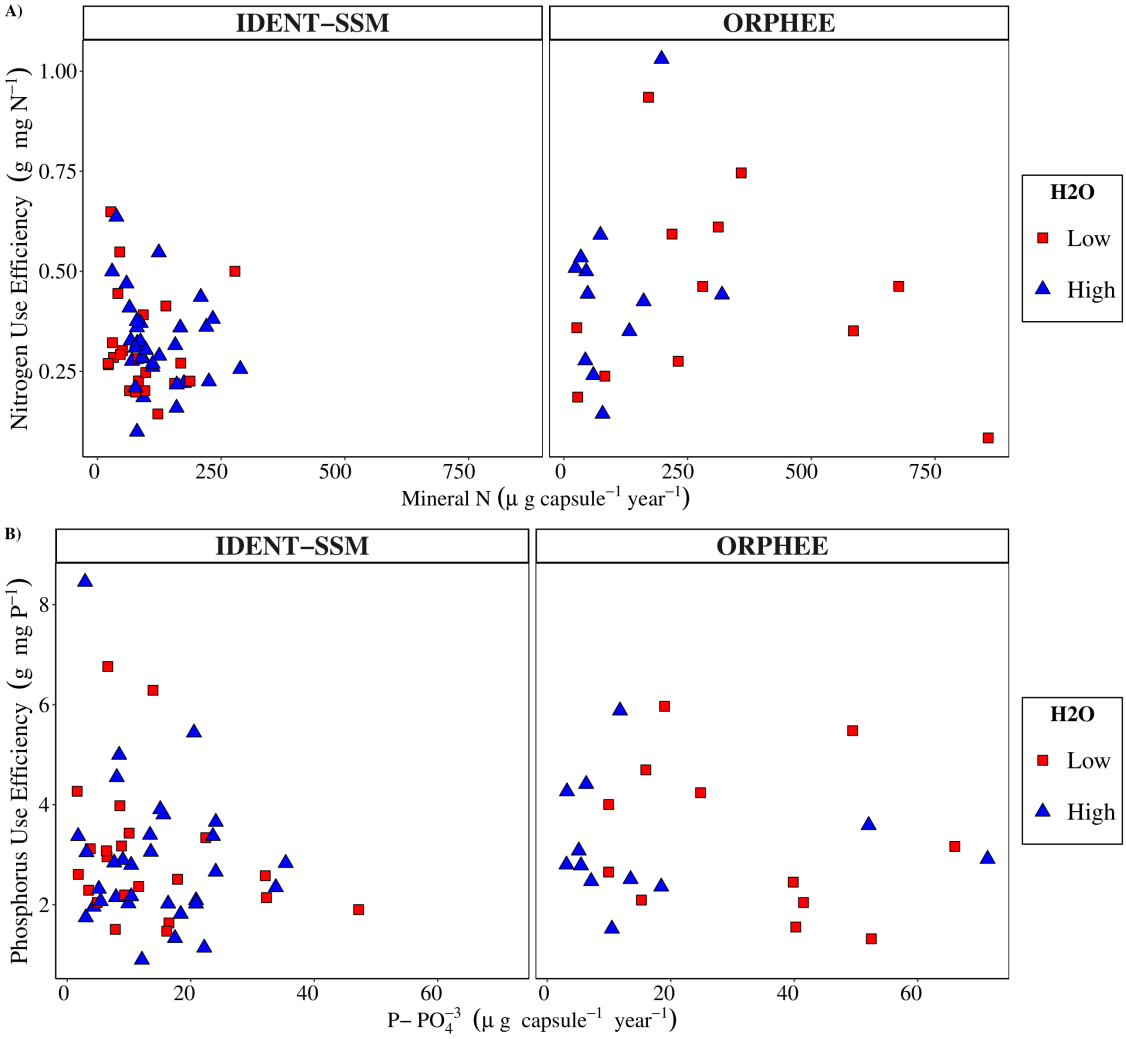

Figure S4.2: Linear correlation between mean soil mineral N (sum of NH_4^+ and NO_3^-) and phosphate availability and plot-level N and P use efficiency at the IDENT-SSM and ORPHEE sites. A) Mineral N (sum of NH_4^+ and NO_3^-) *vs.* nitrogen use efficiency and B) phosphate ($P - PO_4^{-3}$) vs phosphorus use efficiency. Data points are the mean value from IER resins per plot (n = 3 resins per plot at IDENT, n = 4 per plot at ORPHEE) as there was 1 value of NutUE per plot. No significant correlation was found for nutrient at either site.

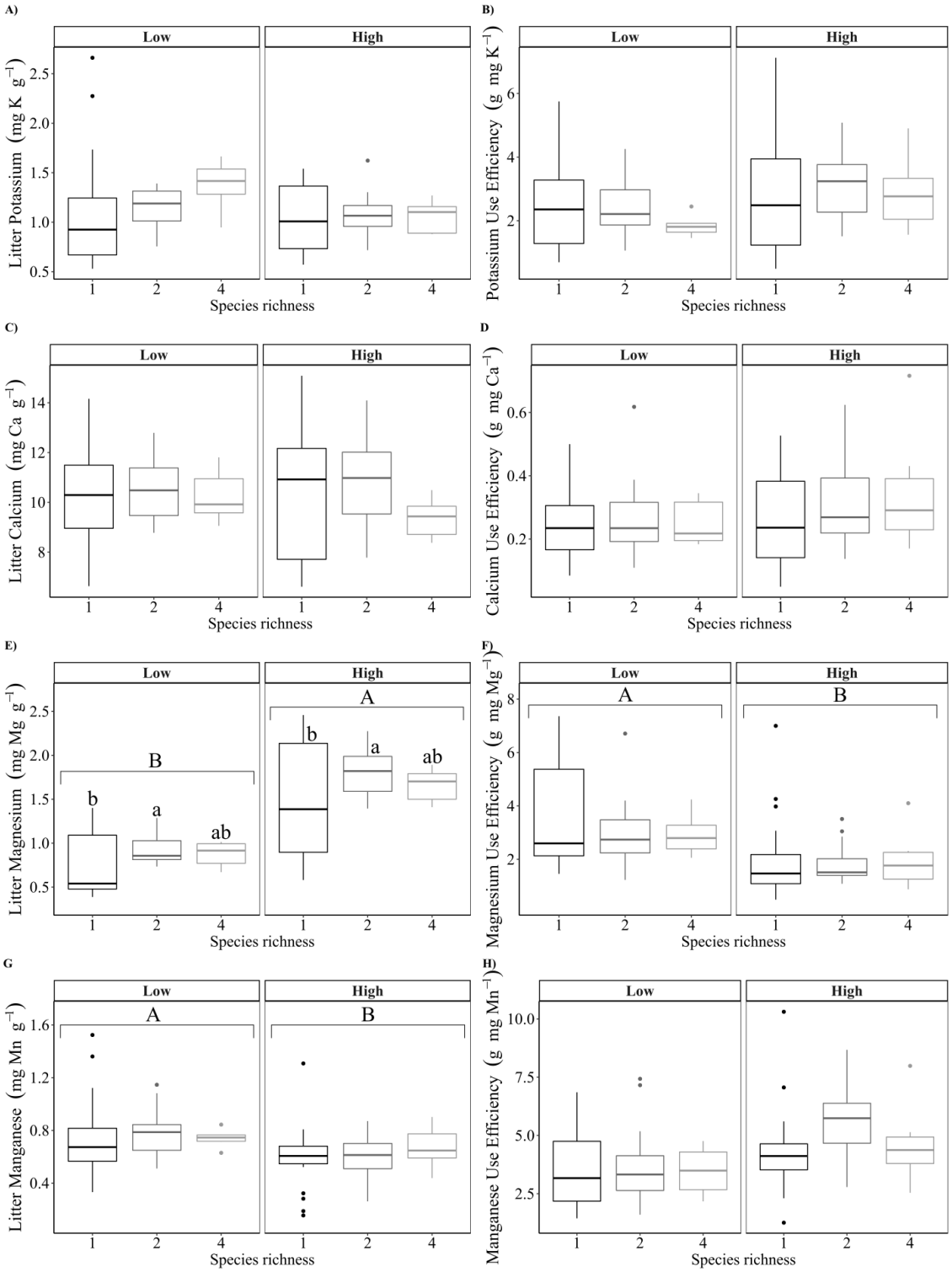

Figure S4.3: Box and stem plots showing the effect of tree species richness (1, 2 or 4 species) and water availability treatment (low or high H_2O) on A) litter potassium concentration, B) potassium use efficiency (KUE), C) litter calcium concentration, D) calcium use efficiency (CaUE), E) litter magnesium concentration, F) magnesium use efficiency (MgUE), G) litter manganese concentration, and H) manganese use efficiency (MnUE) at the IDENT-SSM site. Pairs of panels lacking letters indicate no overall effect (Table 4.1). SR means with different lowercase letters within an H_2O treatment are significantly different ($p < 0.05$, Tukey's HSD test). Significant overall H_2O treatments are indicated by uppercase letters ($p < 0.05$).

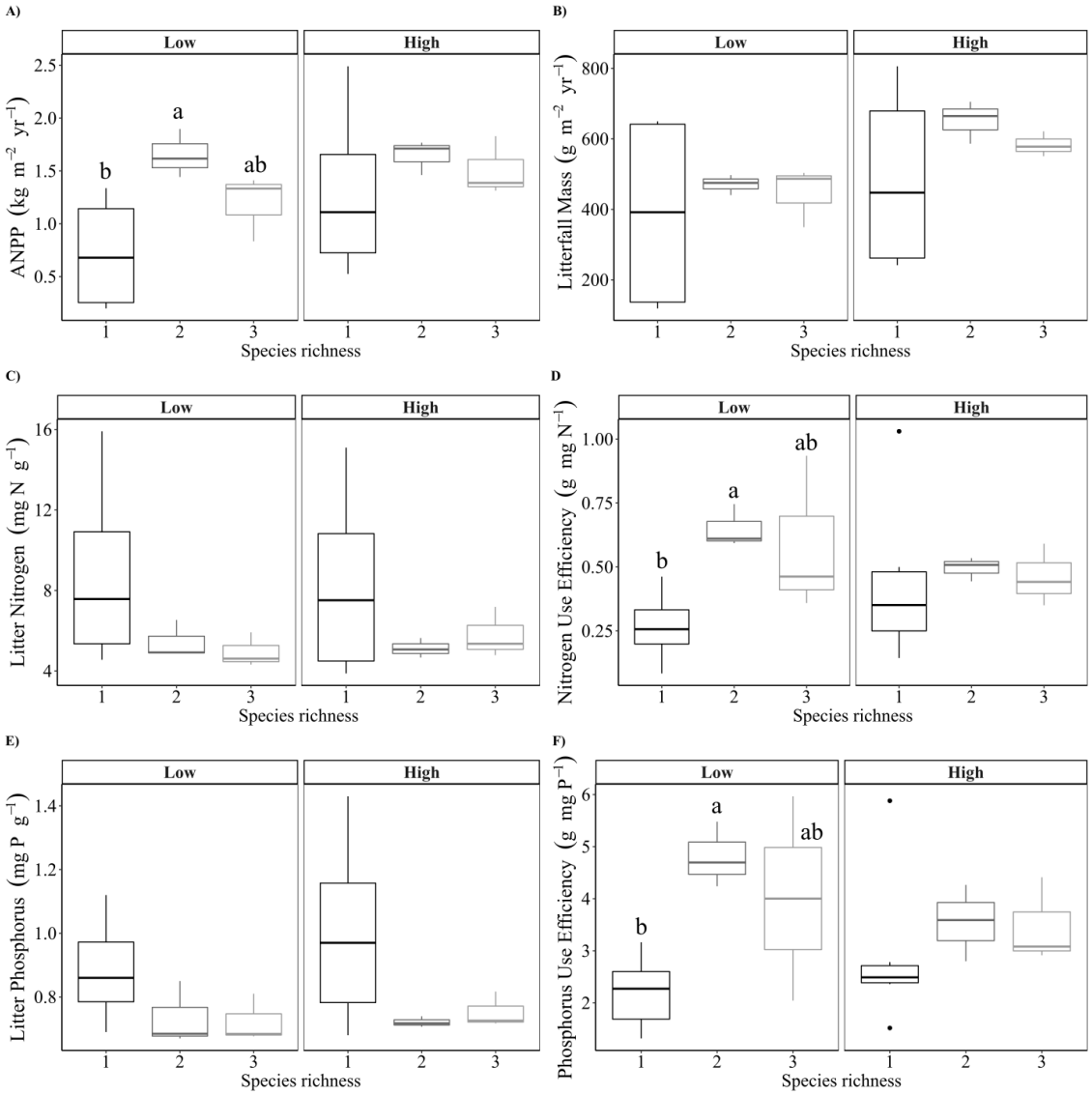

Figure S4.4: Box and stem plots showing the effect of tree species richness (1, 2 or 3 species) and water availability treatment (low or high H_2O) on A) ANPP, B) litterfall biomass, C) litter nitrogen concentration, D) nitrogen use efficiency (NUE), E) litter phosphorus concentration, and F) phosphorus use efficiency (PUE) at the ORPHEE site. Pairs of panels lacking letters indicate no overall effect (Table 4.1). SR means with different lowercase letters within an H_2O treatment are significantly different ($p < 0.05$, Tukey's HSD test).

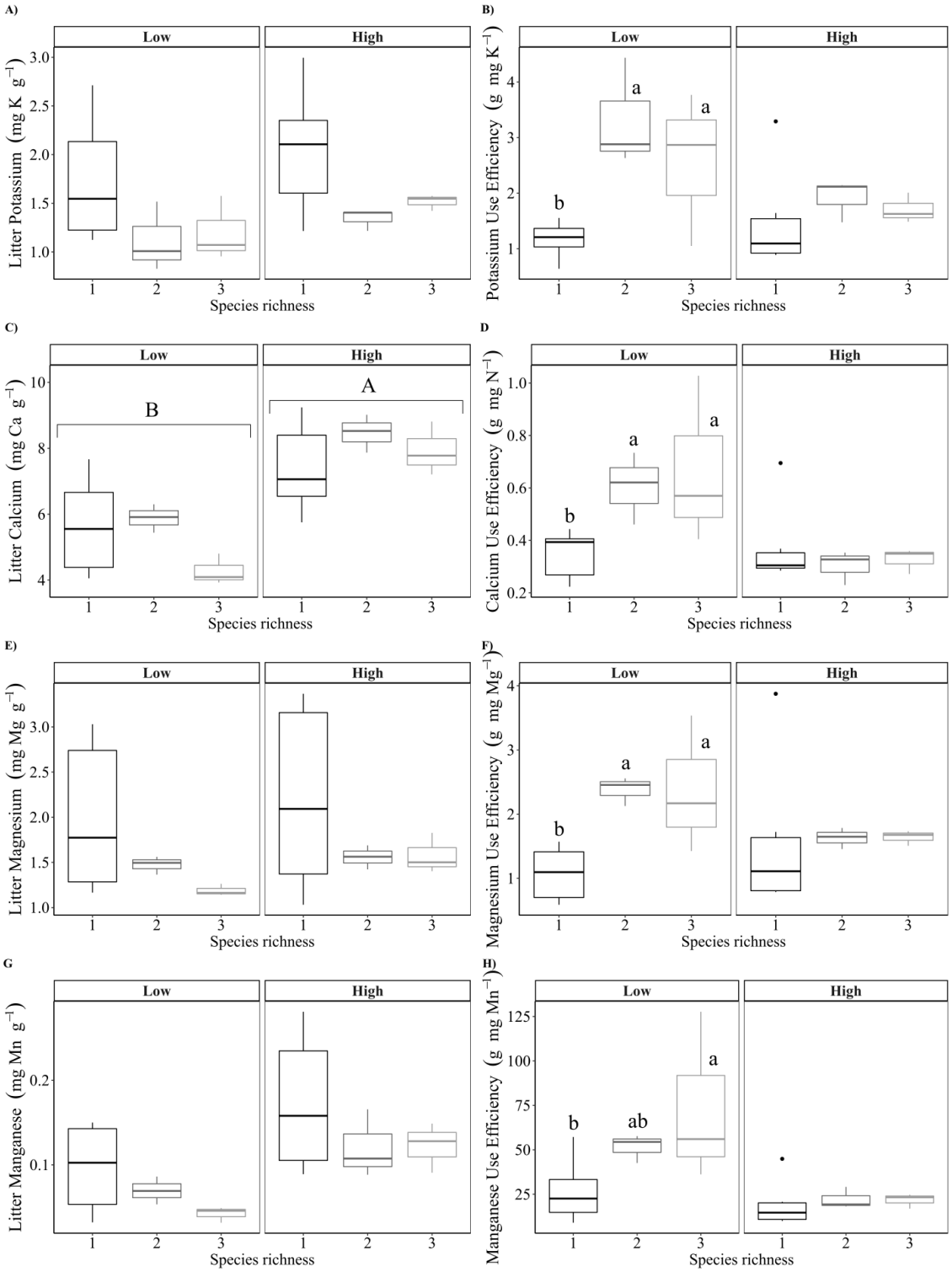

Figure S4.5: Box and stem plots showing the effect of tree species richness (1, 2 or 3 species) and water availability treatment (low or high H_2O) on A) litter potassium concentration, B) potassium use efficiency (KUE), C) litter calcium concentration, D) calcium use efficiency (CaUE), E) litter magnesium concentration, F) magnesium use efficiency (MgUE), G) litter manganese concentration, and H) manganese use efficiency (MnUE) at the ORPHEE site. Pairs of panels lacking letters indicate no overall effect (Table 4.1). SR means with different lowercase letters within an H_2O treatment are significantly different ($p < 0.05$, Tukey's HSD test). Significant overall H_2O treatments are indicated by uppercase letters ($p < 0.05$).

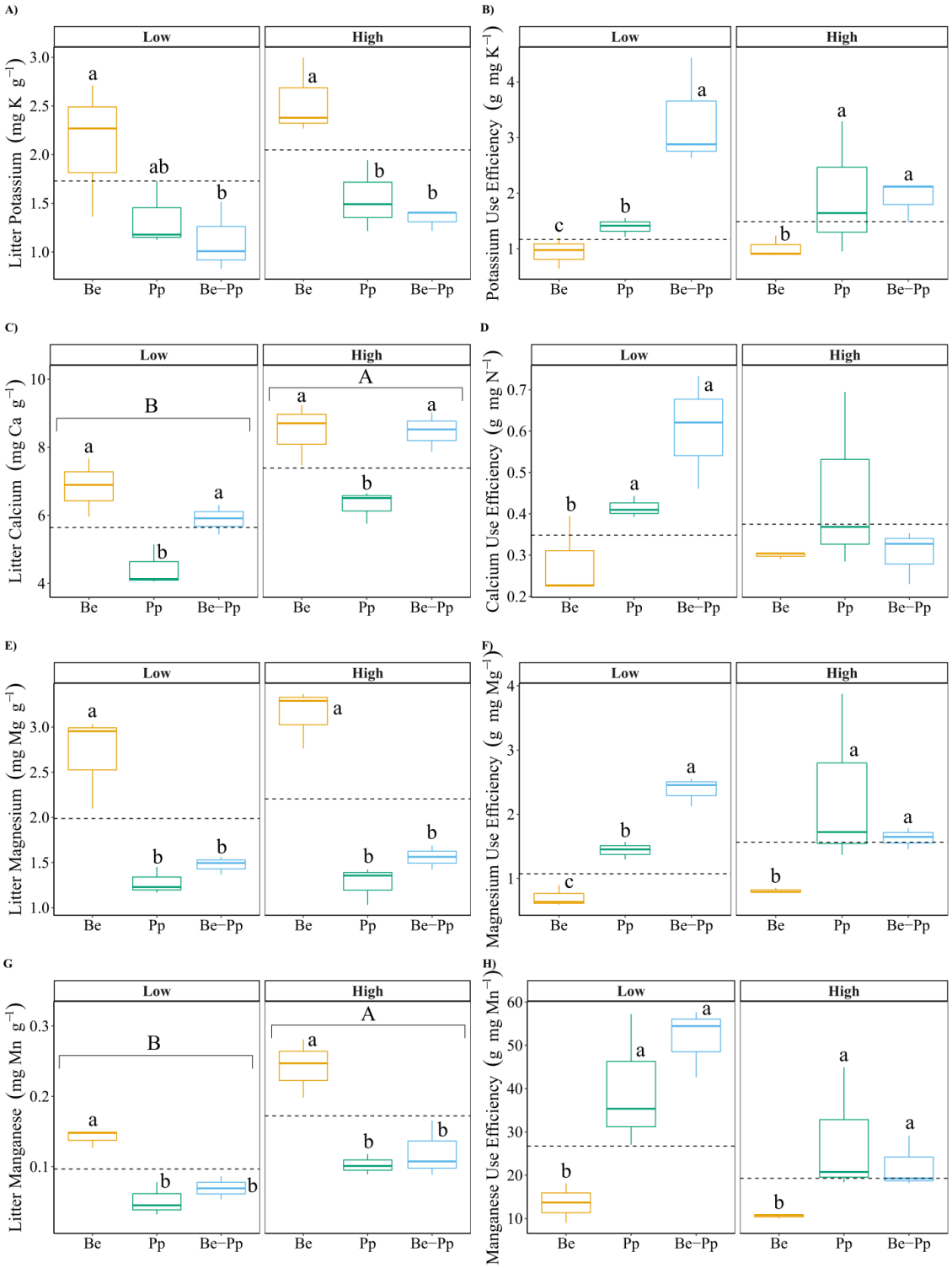

Figure S4.6: Box and stem plots showing the effects of species identity and water availability treatment (low or high H_2O) on A) litter potassium concentration, B) potassium use efficiency (KUE), C) litter calcium concentration, D) calcium use efficiency (CaUE), E) litter magnesium concentration, F) magnesium use efficiency (MgUE), G) litter manganese concentration, and H) manganese use efficiency (MnUE) for *Betula pendula* (Be), *Pinus pinaster* (Pp), and Be-Pp mixtures at the ORPHEE site. The horizontal dashed line represents the expected value for a 50% Be-Pp mixture value. Pairs of panels lacking letters indicate no overall effect (Table 4.1). SR means with different lowercase letters within an H_2O treatment are significantly different ($p < 0.05$, Tukey's HSD test). Significant overall H_2O treatments are indicated by uppercase letters ($p < 0.05$).

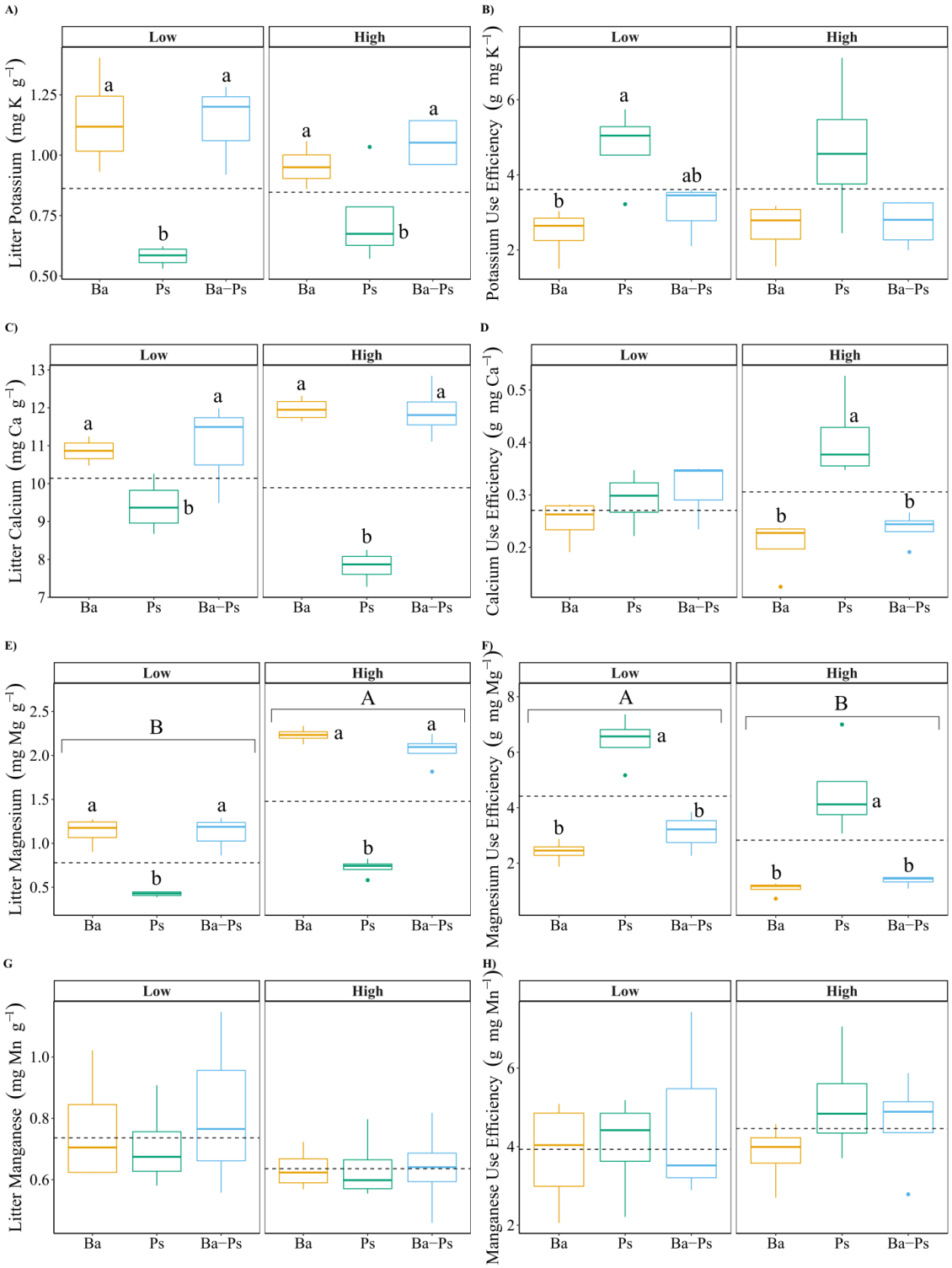

Figure S4.7: Box and stem plots showing the effects of species identity and water availability treatment (low or high H_2O) on A) litter potassium concentration, B) potassium use efficiency (KUE), C) litter calcium concentration, D) calcium use efficiency (CaUE), E) litter magnesium concentration, F) magnesium use efficiency (MgUE), G) litter manganese concentration, and H) manganese use efficiency (MnUE) for *Betula papyrifera* (Ba), *Pinus strobus* (Ps), and Ba-Ps mixtures at the IDENT-SSM site. The horizontal dashed line represents the expected value for a 50% Ba-Ps mixture value. Pairs of panels lacking letters indicate no overall effect (Table 4.1). SR means with different lowercase letters within an H_2O treatment are significantly different ($p < 0.05$, Tukey's HSD test). Significant overall H_2O treatments are indicated by uppercase letters ($p < 0.05$).

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General discussion

Increasing tree diversity is a long-term management strategy to mitigate the negative effects of global change (Vilà-Cabrera et al. 2018), by increasing resistance and resilience of forests to disturbances, such as drought (Lebourgeois et al. 2013; Pretzsch et al. 2013b). This diversity effect is due to asynchrony and complementarity (shade, nutrient content, water use) among different tree species (Jucker et al. 2014a; Kardol et al. 2018; Morin et al. 2014). However, most previous studies have focused on aboveground diversity effects, while belowground effects have received less attention, especially in the context of climate change. More specifically, various steps of the nutrient cycle have seldom been studied concurrently.

The purpose of this thesis was thus to investigate the interaction between tree diversity and water availability on nutrient cycling in forests. More precisely, we tested whether changes in water availability modify potential tree diversity effects on several key parts of nutrient cycling. To do so, we used two experimental platforms, manipulating both tree diversity and water availability (*via* irrigation and partial rain exclusion) in temperate forest regions (ORPHEE, southwestern France and IDENT-SSM, northern Ontario, Canada). Overall, some of our hypotheses were satisfied, while others were not validated (Table D.5). However, we can synthesize the following results from the chapters of this thesis (Fig. D.1): when looking at belowground nutrient cycling, we found that processes affecting organic matter decomposition (the breakdown of organic matter by extracellular enzymes and protein depolymerization) were positively influenced by higher water availability in the topsoil (Chapter 1 and Chapter 2), while net mineralization was negatively impacted by increasing water availability, due to its positive effect on nutrient immobilization (Chapter 3). Mixing tree species had a positive effect on microbial activity in the mid-soil layer (Chapter 1), and also led to asynchrony in nutrient uptake at budburst (Chapter 3). As for aboveground nutrient use, we found that species identity (i.e. the species present in a stand) was a stronger driver than species diversity (Chapter 4).

The different sections of this general discussion are organized according to Figure D.1. First, belowground processes are detailed, which were studied at various spatial and temporal scales. Focusing on the topsoil, the biogeochemistry was driven by water

availability, which also influenced the link between litterfall and forest floor nutrient concentrations. No temporal complementarity on soil nutrient availability was revealed at this soil layer, but the mid-soil layer activity was increased when mixing tree species. In order to discuss nutrient uptake and use efficiency, the 'Aboveground processes' section is divided into seasons: winter, spring, summer and fall. The conclusion summarizes the principal findings, the lack of interactive effects, and its implication for ecosystem functioning. Finally, limitations to the study and further perspectives are discussed.

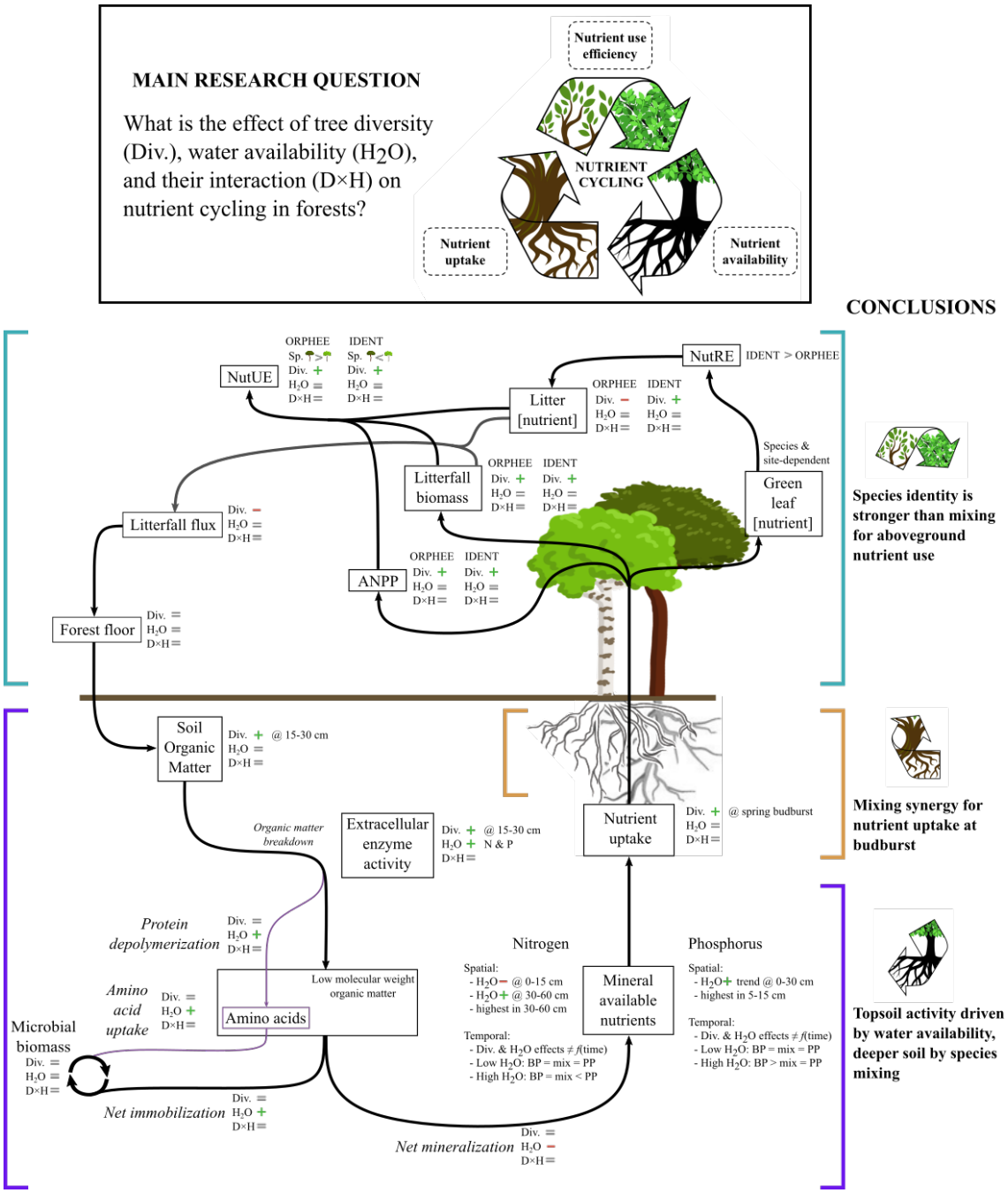

Figure D.1: Diagram presenting the main results and conclusions of the thesis.

Table D.5: Results of the hypotheses for each chapter of the thesis.

Chapter	Hypothesis	Result
I. Spatial complementarity of soil nutrient availability	$H_{1.1}$: Positive effect of mixing on soil enzyme activity, especially in topsoil	✓ (only in midsoil) [Figure 1.3]
	$H_{1.2}$: Positive effect of water availability on soil enzyme activity	✓ [Figure 1.5]
	$H_{1.3}$: Stronger effect of mixing under low water availability	✗ No interactive effect [Table 1.2]
II. Soil organic nitrogen breakdown	$H_{2.1}$: Mixing leads to increase in gross organic N rates due to an increase in microbial biomass	✗ [Figure S2.1]
	$H_{2.2}$: Positive effect of water availability on rates	✓ [Figure 2.3]
	$H_{2.3}$: Stronger effect of mixing under low water availability	✗ (trends emerged) [Figure 2.3]
III. Temporal complementarity of soil nutrient availability and nutrient uptake	$H_{3.1}$: Higher mineralization and soil nutrients in mixed stands, especially during growing period	✗ [Figure 3.3, Figure 3.4]
	$H_{3.2}$: Asynchrony in nutrient uptake between birch and pine strongest at budburst	✓ [Figure 3.5]
	$H_{3.3}$: Positive effect of high water availability on all process rates	✗ [Figures 3.3, 3.4 & 3.5]
4. Nutrient use efficiency (NutUE)	$H_{4.1}$: Negative effect of mixing on NutUE	✗ [Table 4.1, Figures 4.5, Figure S4.4]
	$H_{4.2}$: Positive effect of high water availability on NutUE	✗ [Table 4.1, Figures 4.5, Figure S4.4]
	$H_{4.3}$: Negative effect of mixing stronger under low water availability	✗ [Table 4.1, Figures 4.5, Figure S4.4]

1 BELOWGROUND PROCESSES

1.1 Topsoil activity driven by water availability

In addition to the analyses by chapter, we looked closely at the organic matter dynamics prior to mineralization, to understand how tree species composition and water availability could drive belowground processes. Both forest floor and mineral soil along the soil profile were analyzed for organic carbon (C), the results of which were presented in a companion paper, Fanin et al. 2021. The total C stocks were calculated using equivalent soil mass for each soil layer separately, using a cubic spline interpolation (Wendt and Hauser 2013). From this data, total organic matter was estimated by multiplying organic C by a factor of 2 (Pribyl 2010). When looking at the forest floor and mineral topsoil, there was no effect of mixing on the total organic matter. This lack of species mixing effect in the forest floor and in the top soil layers was previously reported for pine & deciduous (beech or oak) mixtures in Europe (Osei et al. 2021). While annual litterfall mass at our site increased with mixing (Fig. 4.5, Fig. S4.4), most of the litter remained intact on the soil floor. Differences in decomposition rates and soil microorganism activity had likely not developed in the mixed species plantation, as indicated in Fanin et al. 2021 and in the results of Chapter 2 (Fig. S2.1). Thus, we did not observe higher organic matter in the forest floor (partially decomposed litter) and mineral soil horizons.

Linking litterfall to forest floor and soil nutrient dynamics, we found a relationship between litterfall and forest floor nutrient concentrations (Fig. D.2), which was lost when looking at the mineral soil. Mixing two contrasting species, a deciduous broadleaf (birch) and an evergreen coniferous (pine) at ORPHEE, led to higher litterfall mass, although this litter had a lower concentration of nutrients due to the dominance of the nutrient-poor pine litter. The increase in organic matter *via* litterfall did not influence total organic matter in the forest floor, but the trends of forest floor nutrient concentrations were similar to those found in litter nutrient concentrations (Fig. D.2). In general, birch litter and forest floor had significantly higher total nitrogen (N), which is logical, given that birch leaves are higher in N than pine (van den Burg 1985). However, this effect of birch's higher nutrient concentration was not observed in the topsoil mineral layers, where we found no significant effect of plot composition on total N (Annex E.1). This may be due to the low overall nutrient concentration in the mineral soil compared to the forest floor (i.e. forest floor total N 8.18 ± 0.47 *vs.* topsoil total N 1.16 ± 0.09 mg N g^{-1}), or due to other microbial limitations that may not lead to higher organic matter decomposition and N acquisition in the birch plots. Although the positive effect of mixing on litterfall mass was not matched by an increase in forest floor organic matter, this may be because the latter was more strongly influenced by water availability.

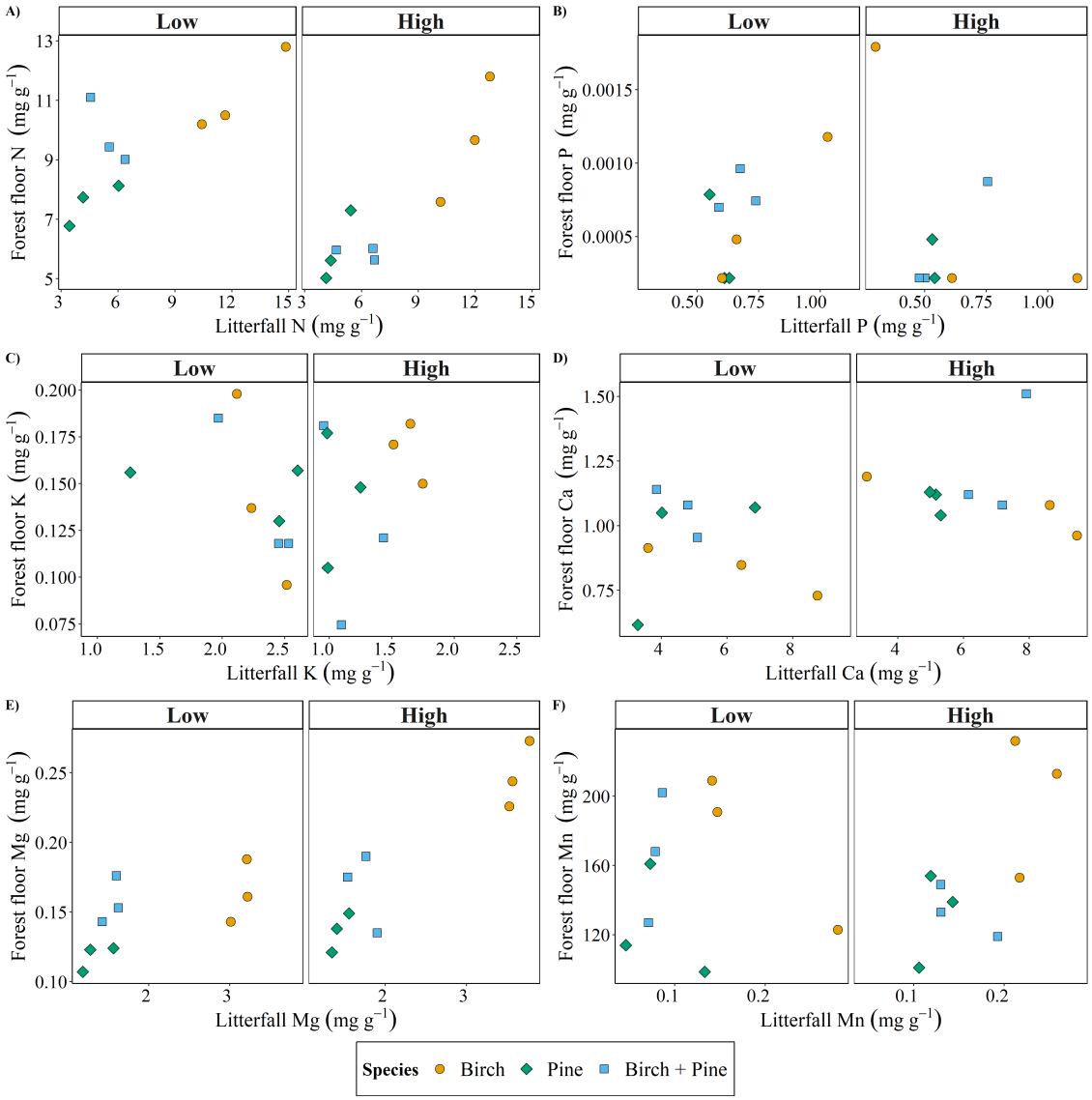

Figure D.2: Relationship between litterfall and forest floor nutrient concentrations for pure birch (*yellow*, circles), pure pine (*green*, diamonds), and mixed birch-pine (*blue*, squares) under both low and high water availability at ORPHEE. Samples for the forest floor were taken in March 2018 and the litterfall in the autumn of 2018.

Instead, the higher water availability, stimulated by seasonal irrigation, had a stronger impact on the organic matter, as well as belowground processes on both the forest floor and topsoil (0-15 cm). Interestingly, high water availability decreased the organic matter in the forest floor by 43% on average, while it led to significantly higher soil organic matter in both the 0-5 and 5-15 cm soil layers, by 30 and 45%, respectively (Fig. D.3). This indicates that increased water availability likely increased the rate of decomposition processes (Fanin et al. 2020; Zhong et al. 2017) by favoring microbial activity and processing in the topsoil (Brockett et al. 2012; Cisneros-Dozal et al. 2007). This then led to higher amounts of soil organic matter that could then be broken down into lower molecular weight organic matter. One of the ways to study this breakdown is *via* soil extracellular enzyme activities, which are released by soil microorganisms to acquire energy and resources (Burns 1982; Burns

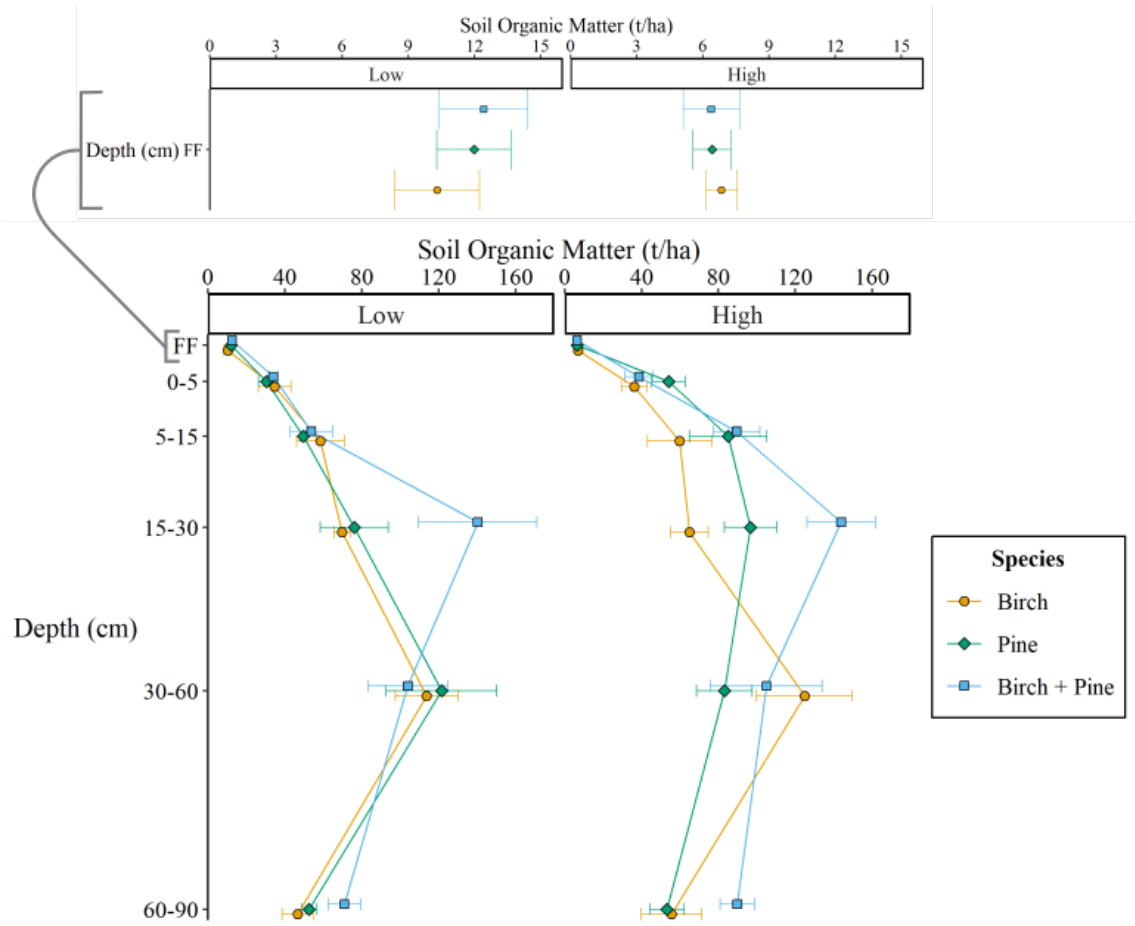

Figure D.3: Soil organic matter (t ha^{-1}) along the soil profile, with a zoom on the forest floor stocks. Data are presented for pure birch (*yellow*, circles), pure pine (*green*, diamonds), and mixed birch-pine (*blue*, squares) under both low and high water availability at ORPHEE. Samples were taken in March 2018.

et al. 2013), although roots have also been found to release enzymes to improve nutrient acquisition (Duff et al. 1994; Gartner et al. 2012; Godlewski and Adamczyk 2007). In our chapter one study (Maxwell et al. 2020a), we found that irrigation also significantly increased N- and P- related enzyme activities, by 35 and 27% in the 0-5 cm layer, and by 24 and 16% in the 5-15 cm soil layer, respectively (Fig. 1.5).

More specific to N, protein depolymerization is a key step to belowground N cycling (Schimel and Bennett 2004; Schulten and Schnitzer 1998), as it breaks down proteins to amino acids, which can then be mineralized (or immobilized) and made available for uptake. In general, both ammonium and nitrate are readily taken up by plants and microorganisms. In some ecosystems, such as nutrient-poor boreal forests, direct uptake of amino acids by trees is a predominant way of nutrient uptake (Lipson and Näsholm 2001). We found that higher water availability also stimulated these organic N breakdown (protein depolymerization) rates (Fig. 2.3), which were measured on soil samples from the top 0-15 cm layer. Although lower enzyme activities in the low water availability treatments could have been explained by microbial hotspots and thus microbial abundance

and activity (Kuzyakov and Blagodatskaya 2015), microbial biomass was unaffected by the different treatments (Fig. S2.1). Thus, we suggest that enzyme activity and protein depolymerization rates were substrate-limited rather than activity-limited (Geisseler and Horwath 2008; Noll et al. 2019b). Indeed, high water availability increased plant input to the soil, as indicated by the higher litterfall mass and more rapid decomposition (Fanin et al. 2021) in the high water availability treatments (explaining the lower pool of organic matter in the forest floor). This resulted in higher quantities of organic matter in the topsoil and stimulated microbial activity, leading to higher rates of both protein depolymerization and extracellular enzyme activity. Another possibility is that dry episodes (Fig. SS1.1) could have caused a significant change in the composition of root exudates, both during these dry periods and after rewetting (Gargallo-Garriga et al. 2018), leading to lower activity under the low water availability treatment.

The strong positive effect of high water availability on soil organic matter, extracellular enzyme activities, and protein depolymerization rates in the topsoil, along with a null effect of species composition, indicate that water availability may be a stronger driver for topsoil nutrient cycling than species composition. This was also true for the net nutrient mineralization rates, which were negatively impacted by high water availability, i.e. there was a higher net immobilization of mineral nutrients by the soil microorganisms in the irrigated treatment (Fig. 3.4). Nitrogen and P mineralization at ORPHEE followed the same trends, suggesting a stable microbial stoichiometry at our site (Zechmeister-Boltenstern et al. 2015). However, we cannot make conclusions about gross mineralization and immobilization rates during the incubation periods, since these cannot be quantified with the *in situ* soil incubation method we used. Water availability also strongly impacted the availability of mineral nutrients along the soil profile: available N decreased by 40% in irrigated plots in the topsoil (0-15 cm), and increased by 55% in the deeper soil layers (30-90 cm), while it was unaffected by species composition (Table 1.1, Fig. 1.6). The highest quantity of available N extracted from fresh soil was in the 30-60 cm layer, with 2.1 ± 0.2 µg N g^{-1} in low and 2.8 ± 0.4 µg N g^{-1} in high water availability plots. The lower available N in the topsoil is probably due to the strong presence of fine absorbing roots in the upper soil layers (Altinalmazis-Kondylis et al. 2020), which took up any available nutrients, coupled with the leaching of soluble mineral N-forms (NH_4^+ and NO_3^-) into the deeper soil layers. Interestingly, the highest quantity of available P recovered was in the 5-15 cm soil layer, and tended to also increase with irrigation (Fig. 1.6). In general, P availability decreased along the soil profile. The decrease in tree and understory root biomass along the soil profile (Table SI.2), likely reduced the soil acidification *via* root exudates (Nardi et al. 2002) and thus P desorption (Achat et al. 2013b), explaining the declining P availability. Thus, the vertical availability of nutrients seems to be driven by water availability, and differs among soil depths due to root profiles of both the trees and the understory communities.

The strong effect of water availability on topsoil microorganism activity was also demonstrated when investigating temporal complementarity or variation in soil nutrient availability among species composition treatments. The nutrients accumulated in the ion-exchange resins throughout the growing (~May - November) and the dormant (~November - May) periods were negatively affected by high water availability. A combination of higher leaching and higher nutrient demand in these irrigated plots may explain these trends. Indeed, trees with higher water availability had a larger biomass, and may have needed to take up more nutrients (Augusto et al. 2000). Under high water availability, trends for species composition effects on accumulated nutrients throughout both the growing and dormant season emerged: there was slightly higher N in pure pine than in pure birch or mixed birch-pine, and slightly higher P in pure birch than in the other treatments. The accumulation of N could be indicative that the pine trees in their monocultures did not need to take up all available N. Pine trees in monocultures were around 2.5 times larger than the birch trees (Table 3.2), and thus they likely had higher rates of remobilization (Miller and Miller 1987), i.e. the ability to use stored internal N resources for growth and reproduction. As trees develop, the rate of N uptake slows concomitantly with an increase in their potential storage capacity (Miller 1986). The higher soil available P in pure birch could have been due to the higher quality of leaf litter compared to pine. Hardwood species, including birch, are more effective at cycling nutrients, such as P, through foliage and litterfall back to the soil, compared to softwood species such as pine (Achat et al. 2013b; Hobbie 2015; Sherman et al. 2006). However, the temporal measurements (soil nutrients, mineralization) revealed no evidence for a species mixing effect, i.e. we did not observe temporal complementarity between birch and pine, at least not for belowground processes.

As for available mineral N and P, differences emerged both from a spatial and temporal point of view, due to differences in diffusibility within the soil, and in nutrient requirements from the aboveground community. Although roughly similar quantities of N and P were extracted from fresh soil during soil core sampling in March 2018 at different depths (Table S1.3), the accumulated nutrient availability *via* the ion-exchange resins showed 10-fold higher concentrations of N compared to P (Fig. 3.3, Table 4.2). This is likely due to the higher mobility of N versus P in the soil, so N accumulates more in resins than P (Buck et al. 2016; Qian and Schoenau 2002). This was also the case at the second experimental site, IDENT-SSM (Table 4.2). The annual accumulated nutrients were not significantly influenced by the difference in water availability, unlike the negative effect of high water availability at ORPHEE. This may be due to a range of factors, such as differing soil water content or soil types, or the weekly irrigation during three months at IDENT-SSM, versus the daily irrigation during five months at ORPHEE.

1.2 Mid-soil activity driven by species mixing

While the effect of mixing tree species was not detected in the topsoil layers, a small effect of tree species on topsoil enzyme activities was also found in previous studies (Diao et al. 2020; Herold et al. 2014; Weand et al. 2010). Although the different stands had different litterfall and forest floor nutrient concentrations, this did not influence differences in organic matter breakdown dynamics in the topsoil. The high biomass of both tree and understory roots in the top 0-15 cm soils layers (Table S1.2) may have led to a maximum of microbial activity and competition in this layer (Kuzyakov and Xu 2013). This competition for nutrients, and nutrient limitation, was similar among species compositions, as indicated by similar enzyme activities.

However, strong mixing effects on breakdown activity emerged at the intermediate soil layer (15-30 cm). A strong positive effect of mixing birch and pine on C- and N-related soil extracellular enzyme activity was found in the 15-30 cm soil layer (Fig. 1.3, Fig. 1.4), which we interpreted as a result of higher nutrient demand in the mixed stands. In the birch-pine mixture, overyielding was found for the aboveground net primary productivity (Fig. 4.6), although this was not the case for belowground root standing biomass (Altinalmazis-Kondylis et al. 2020). The higher tree productivity observed in mixed stands could have stimulated the soil microorganism biomass through an increase in root exudate quantity and diversity (Steinauer et al. 2016), or favored C allocation to ectomycorrhizal fungi to obtain N from organic molecules to sustain biomass production (Fernandez and Kennedy 2016; Lindahl and Tunlid 2015). An increase in priming in mixed stands, by acidification and release of organic acids (Blagodatskaya and Kuzyakov 2008), could have released mineral-associated organic matter, releasing nutrients. Because the ORPHEE study region is known for its limitation in P (Achat et al. 2013b; Trichet et al. 2009), and our observed lack of increase in the P-related enzyme with mixing in this intermediate layer, this implies that the limitation in P was partially lifted and microorganisms were more limited by C and by N, therefore increasing these enzyme activities in this layer (Fatemi et al. 2016).

An increase in microbial biomass due to priming by roots in the mixed stands (Blagodatskaya and Kuzyakov 2008) would also have increased organic matter inputs, as over 80% of soil organic N is estimated to originate from microorganisms (Simpson et al. 2007). This could help explain the increase in soil organic matter at the 15-30 cm soil layer, which increased by 85% in the mixed-species plot compared to either of the monocultures (Fig. D.3). Potential effects at the 30-60 cm soil layer were likely constrained by the presence of the hardpan layer at a depth of around 50 cm (Achat et al. 2008; Fanin et al. 2021). The general steady increase in organic matter up to this point could be due to the decrease in microbial activity along the vertical profile (Agnelli et al. 2004), leading to less overall decomposition of the organic matter in deeper soils. Indeed, almost 50% of soil organic

matter stocks in the topmost meter of soil were found to be in the soil layer between 30 and 100 cm (Balesdent et al. 2018; Jobbágy and Jackson 2001).

1.3 Deeper soil activity unaffected by different treatments

Deeper soil (30 - 90 cm) enzyme activity was low and largely unaffected by the species composition or water availability treatments (Fig. 1.3). Several factors may explain these results. First, this may be due to lower microbial biomass (Agnelli et al. 2004) and lower microbial activity (Fang and Moncrieff 2005) in the deeper soil, where conditions are less favorable for organic matter degradation than in the topsoil. It could also be due to the hard pan, present at around 50 cm in depth, which limited root exploration (Fanin et al. 2021). Finally, it could be that it takes more time to modify deeper soil conditions and activity (Balesdent et al. 2018), and we sampled the soil only ten years after tree plantation.

2 ABOVEGROUND PROCESSES

2.1 Winter

While belowground nutrient cycling was affected by water availability and by mixing birch and pine according to vertical depth, we also looked more closely at the yearly trends to examine the potential effects of these factors on nutrient uptake and aboveground nutrient cycling in the trees. Throughout the dormant period, when temperatures were mild enough, pine trees, the evergreen conifers, have a small amount of photosynthetic activity due to the cohort of needles which stay on the tree throughout winter (Freeland 1944; Medlyn et al. 2002). This is true in regions where temperatures are mild enough during the winter to allow this activity, and therefore not in very cold regions. By definition, deciduous broadleaf trees are leafless throughout the dormant period and stay relatively inactive. This difference in potential winter activity may have partially led to the advantage of pine in the birch-pine mixture at the ORPHEE site in southwestern France, but to a disadvantage at the IDENT-SSM site in northern Ontario, Canada. The plantation density and age likely led to the differences in growth patterns (Fig. 4.6, Fig. 4.7).

As mentioned in the previous section, ion-exchange resins were installed during the growing and dormant periods to assess soil nutrient availability. Although the dormant period dates coincide with early spring, the results suggest that soil nutrient availability was not negligible in winter.

2.2 Spring

The full canopy in the evergreen trees gives these species an advantage at the beginning of spring, when microorganism activity is stimulated with the increasing temperature, giving these trees the potential to have the first access for the available nutrients. At this time, the deciduous broadleaves have a strong need to develop their leaves to maximize the surface area for photosynthesis and to take up nutrients *via* transpiration and the soil-water-air continuum. Thus, mixing birch and pine, a deciduous broadleaf and an evergreen conifer, could lead to interesting dynamics for nutrient uptake around spring budburst time.

The ^{15}N soil labeling experiment at ORPHEE revealed interesting patterns at this key phenological point. In the early spring, birch trees in monoculture had full access to the canopy space, and were able to start bud swelling and budburst when the temperature and light conditions were appropriate. In the mixture, however, the stand age and density led to pine overtaking birch for productivity in the mixture (Morin et al. 2020). Therefore, at the time of the soil labeling experiment in March, birch trees were largely overshadowed by pine, which had occupied most of the canopy space (Martin-Blangy et al. submitted), leading to delayed budburst, as previously found for oak mixed with pine (Perot et al. 2021). While nutrients can be absorbed into the tree prior to leaf swelling and budburst (Bazot et al. 2016; Maxwell et al. 2020b), pine trees had a bigger advantage by already having a full canopy cover with the previous cohorts' needles, and conifers tend to start transpiration and nutrient uptake prior to the leaf growth of broadleaf trees (Rothe 1997). Interestingly, pine in the mixture took up more than in the monoculture (Fig. 3.5), indicating that it took advantage of birch's later budburst. This could indicate both a release of intraspecific competition for pine, in combination with an increase in interspecific competition for birch in the mixture – i.e. a win-lose situation (Forrester et al. 2011; Nanami et al. 2011; Vanclay et al. 2013).

Pine's increase in uptake at the detriment of birch at budburst at ORPHEE was closely connected to the stand structure (Rötzer et al. 2004), such as the density, tree height, canopy cover, and understory biomass. While we did not look into nutrient uptake patterns at IDENT-SSM in Canada, we can deduce the dynamics from the aboveground canopy structure. In the younger and denser birch-pine mixture at IDENT-SSM, the aboveground canopy was largely dominated by birch over pine (Fig. 4.7). Thus, we would not expect there to be an antagonistic effect of mixing on birch budburst uptake in the

mixture. Because pine also greatly reduced its productivity in the mixture compared to the monoculture (Fig. 4.7), including its litterfall mass, which is strongly related to its canopy cover (Staelens et al. 2004), it could have a weaker nutrient uptake at budburst.

Overall, when using the soil-applied ^{15}N label, we did not find a significant effect of water availability on the nutrient uptake capacities of the species, but this may have been because the spring labeling experiment took place prior to irrigation being switched on. It also implies that there was no legacy effect of reduced water availability in the control blocks. This is likely because, contrary to previous studies finding an increased allocation to roots with drier conditions (Bakker et al. 2006; Bello et al. 2019), our site did not exhibit strong differences in root biomass between control and irrigated blocks (Altinalmazis-Kondylis et al. 2020). There was also no interaction effect between mixing and water availability, i.e. there was no difference in the effect of species mixing between the high and low water availability treatments. However, indirect effects of water availability on tree growth has the potential to change canopy dynamics in the future, and thus nutrient uptake abilities at budburst.

2.3 Summer

We found that the phenological differences between the two species were important to consider when investigating mixing effects on nutrient uptake at budburst, and that these differences can also be affected by the stand structure. At peak summer vegetation, however, the potential mixing effects were lost (Fig. 3.5). At this period in early June, both trees had reached their maximum leaf canopy, and with it their maximum uptake capacities prior to the unfavorable dry conditions in peak summer (Gholz et al. 1990). Similar to the budburst uptake soil labeling experiment, there was no effect of water availability or its interaction with tree diversity on summer nutrient uptake dynamics (Fig. 3.5).

Nutrients that are taken up throughout the vegetation season are transported to the leaves and throughout the tree for growth and other functioning (Marschner 2012). Nutrients in the green leaves can give us an idea of the nutrient status of trees and of the overall stand. At the ORPHEE experimental site, green leaf nutrient concentrations were on the cusp of adequate N and P concentrations for these species (CSIRO 1997; van den Burg 1985). In contrast, species at IDENT-SSM exhibited green leaf nutrient concentrations within their optimum nutrition ranges. This suggests that the nutrient limitation was stronger at ORPHEE, as trees had a lower nutrient status at this site. Indeed, a widespread severe limitation in P has been previously found in the sandy soils of the *Landes de Gascogne* region of southwestern France (Achat et al. 2013b; Trichet et al. 2009). However, although more nutrients were recovered in the yearly accumulated nutrients at ORPHEE, this is because soil microorganism activity was more spread out into the tree dormant period:

once trees had slowed down nutrient uptake (or almost stopped, once the leaves of the broadleaf deciduous had fallen off), soil microorganisms continued to process organic matter, releasing mineral nutrients into the soil that could not be taken up by the dormant trees (Gallardo and Schlesinger 1994).

2.4 Fall

Fall marks the end of the tree growing period, when trees lose their leaves and needles, and when aboveground nutrient use efficiency can be determined. In general, we found that species identity was a stronger driver than species mixing for these aboveground dynamics. Additionally, differences in water availability played little role in these dynamics.

Prior to leaf abscission in the fall, nutrients are retranslocated from the leaves into other compartments of the tree for storage (Aerts 1996). Because trees growing in soils with higher nutrient availability have been found to have lower retranslocation rates (Achat et al. 2018; Wright and Westoby 2003), we expected diversity effects to lead to higher nutrient availability and lower retranslocation, generating higher nutrient concentration in the leaf litter. However, as we did not find an effect of mixing on nutrient availability, increasing tree species also did not have subsequent effects on retranslocation and litter nutrient concentrations at either of the experimental sites. There was no relationship between soil nutrient availability and nutrient resorption efficiency (NutRE) (Annex E.2). This could be because the stands are still young and have relatively high NutRE values useful for the early years of growth (Colin-Belgrand et al. 1996), and have not yet adapted the NutRE to differences in soil nutrient availability. Species identity, rather than species mixing, was a stronger driver for NutRE at both the IDENT-SSM and ORPHEE sites, although large variations at ORPHEE often led to non-significant results (Fig. 4.8). There was no significant effect of mixing on NutRE values, so potential differences in stand-level litter nutrient concentrations would be due to differences in aboveground nutrient use efficiency or nutrient uptake.

At the end of the growing season, leaves start to fall, and differences in productivity between the treatments could be quantified. Productivity trends within the mixture (both Aboveground Net Primary Productivity [ANPP] and annual litterfall mass) were due to the presence of certain species. At both the ORPHEE and IDENT-SSM sites, litterfall mass trends generally followed those of ANPP (Fig. 4.5, Fig. S4.4), indicating proportional investments into the growth of the leaf canopy among the species and treatments. The species driving the productivity trends at each site varied, due to the difference in stand age and structure. At the younger and denser sites of IDENT-SSM, birch trees, a resource acquisitive and pioneer species (typical of early vegetation succession), was able to grow more rapidly and dominated the small plot canopy space at IDENT-SSM after 6

years of growth (Belluau et al. 2021). This led to higher levels of productivity in the birch-pine mixture, due to birch's ability to take up even more canopy space than in its monoculture with the gaps in the canopy from the slower growing pine. At ORPHEE, birch also performed better in height growth than pine in the first four years of growth (Morin et al. 2020), after which pine growth overtook that of birch, dominating the productivity and canopy packing in mixtures (Martin-Blangy et al. submitted), leading to a proportion of around 90% of the total litterfall mass. Interestingly, these dynamics were not affected by water availability. Even with lower available water, pine's aboveground canopy development was not slowed at ORPHEE, as was birch's at IDENT-SSM. The important effect of species composition on aboveground productivity trends, had further impacts on nutrient use efficiency in the stands.

Stand-level litter nutrient concentrations also strongly depended on the proportion of each species in the overall litterfall and the nutrient resorption efficiency of each. At IDENT-SSM, this meant that, in general, litter nutrient concentration increased in the mixtures, due to the contribution of nutrient-rich litter, and at ORPHEE, litter nutrient concentration decreased in mixtures due to the strong contribution of pine. More specifically, in the birch-pine mixture, this litterfall mass ratio was 3:1 (birch:pine) at IDENT-SSM, and 1:9 at ORPHEE. Therefore, both N and P (along with K, Ca, Mg and Mn) litter concentrations at the stand-levels in the two-species mixture was more influenced by the dominating species. The mixing effect was even stronger at ORPHEE: not only was there a higher contribution of poor nutrient litter from pine, but both birch and pine increased their resorption efficiencies in the mixture, leading to lower litter nutrient concentrations (Fig. 4.9). Similarly to the productivity measurements, there was no effect of water availability on these trends.

The ratio of ANPP to the amount of litterfall nutrients (i.e. the product of litterfall mass and litter nutrient concentration) was used to calculate nutrient use efficiency (NutUE). The strong negative effect of mixing on litter nutrients at ORPHEE, along with an increase in productivity, led to a strong synergistic effect of mixing birch and pine on NutUE (Fig. 4.6). While there was a positive effect of mixing on litter nutrients at IDENT-SSM, this effect was not strong enough within the NutUE to reveal an effect of mixing at this site. Instead, an additive effect of mixing was found (Fig. 4.7). These trends were due to the different species dominance at each site: at ORPHEE, pine dominated the canopy, growth, and the litterfall flux, while the younger denser stands at IDENT-SSM let the ressource acquisitive birch take the lead and dominate over the smaller pine trees. At both sites, water availability did not have an effect on NutUE, nor did it interact with species composition. Thus, aboveground nutrient use was more influenced by species identity than by water availability.

Conclusion

Results synthesis

The influence of mixing tree species effects on nutrient cycling has been reviewed by Richards et al. 2010, and various studies have looked into diversity effects on various steps of the nutrient cycle, as presented in the introduction (Section A.3). The influence of water availability on tree nutrition was reviewed by Kreuzwieser and Gessler 2010, and more specifically the effect of drought on biogeochemical cycling in forests by Schlesinger et al. 2016 (Section A.4). While some studies have looked at various aspects of the interactive effects of tree diversity and water availability in forests (Section A.5), our study was the first to our knowledge to investigate these effects in interaction on the different steps of nutrient cycling in forests.

Three main conclusions to this thesis can be highlighted. First, water availability was the strongest driver for belowground nutrient processes in our experimental sites, with low water availability treatments characterized by less organic matter and slower organic matter breakdown, and higher net microbial immobilization. This result has negative implications for nutrient cycling in a future climate, which is expected to have longer and drier periods of reduced water availability. Second, the effect of species composition belowground was both limited spatially and temporally: there was a positive effect of mixing on organic matter and microorganism activity in the mid-soil layer, and there was a win-lose effect of mixing on pine *vs.* birch at budburst nutrient uptake. Finally, species identity was key for aboveground nutrient processes, on which water availability had little effect. Species dominance, influenced by stand age and density, drove productivity and litterfall nutrient fluxes, and ultimately stand-level nutrient use efficiency. Thus, the choice of species within a mixture is important to consider as it can drive aboveground growth dynamics and canopy covering. The spatial and temporal segregation of tree mixing and water availability effects is important to consider when making conclusions on these drivers either belowground or aboveground.

No interactive effects

Contrary to our expectations, many of our hypotheses were invalidated (Table D.5) in our studied field experimental sites, which could be due to several possible reasons. While we aimed to study tree mixing effects, we only looked at two monocultures and one 2-species mixture at ORPHEE for chapters 1, 2 and 3, due to the poor growth of the *Quercus* genus in the other mixtures. We may have seen more interactions if we had looked into belowground processes at IDENT-SSM, where several two- and four-species mixtures are growing. Another constraint for potential mixing effects was the poor soil conditions at each site, which may have limited potential mixing effects. Also, the young age of the tree plantations means that there may not have been enough time elapsed since tree plantation for the mixing effects to be developed, especially belowground. Finally, we may have lacked to observe tree diversity effects simply because this factor is minor compared to other stronger drivers of biogeochemical cycling, such as water availability and species identity.

Although we predicted the impact of tree diversity to be stronger under low water availability, we did not find significant effects of this interaction on the various nutrient cycling steps studied. Belowground, the impacts of tree mixing and water availability were spatially separated: topsoil biogeochemical processes were impacted by water availability, while the mid-soil layer was positively impacted by mixing the two species (birch and pine). Aboveground, neither water availability nor species mixing had a strong effect; instead species identity was the driver for nutrient use efficiency.

The stress-gradient hypothesis suggests that positive mixing effects are stronger in habitats subject to abiotic stress, while competition between species drives productivity in more favorable conditions (Brooker et al. 2008; Coleman et al. 1994). There are studies on tree diversity and water availability which have been in line with this hypothesis, or against it, either by finding opposite trends, or no interactive effects, such as we have. For example, previous studies on aboveground biomass showed that tree diversity may become more beneficial as water limitations increase (Lebourgeois et al. 2013; Pretzsch et al. 2013b; Ratcliffe et al. 2017), due to complementarity mechanisms among the different tree species. Other studies have found the opposite, that biodiversity effects on aboveground biomass production decreased in strength in drought years (Jucker et al. 2016), or that high overyielding due to mixing occurred at wet sites (Belluau et al. 2021; Jactel et al. 2018). Few studies have looked into this interaction belowground. A recent study using the same experimental sites found that under high water availability, species richness increased soil microbial biomass and to a lesser extent, microbial activity, but no effects were found under low water availability (Strukelj et al. 2021). In contrast to these results, we found no effect of mixing on soil microbial biomass at ORPHEE (Fig. S2.1), although this may be because

we only looked at one 2-species mixture. We did find, however, a tendency for higher water availability to lead to positive mixing effects on gross protein depolymerization rates (Fig. 2.3), similar to the positive mixing effects being found only under high water availability by Strukelj et al. 2021. Another recent study in a natural boreal forest in Canada did find positive tree mixing effects on soil microbial biomass under low water availability (Chen et al. 2019b). Contrary to these results, we did not find a significant interactive effect of mixing birch and pine on belowground processes. This suggests that increasing tree diversity may not be able to offset negative effects of reduced water availability on belowground nutrient cycling. In conclusion, the lack of interactive effect between tree diversity and water availability on nutrient cycling suggests the following: reduced water availability had negative effects on belowground nutrient cycling that could not be offset by increasing tree diversity, but it did not affect aboveground nutrient use, which were stable to differences in water availability.

Implications

There are several implications of these findings for ecosystem functioning. First, a reduction in water availability, as predicted in future climate scenarios, may decrease belowground microbial activity and limit the diffusion of nutrients within the soil, impacting the availability of nutrients for uptake by trees. This reduction in soil water could also maintain nutrients in upper soil layers, which could otherwise be leached with an increase in precipitation. In general, the negative effect of low water availability on microbial activity is likely to slow down nutrient cycling, by reducing the degradation of soil organic matter, which could have further negative impacts on tree productivity. Because we did not find mixing effects in the topsoil, this suggests that mixing tree species may not be able to offset the negative effects of drought for belowground nutrient cycling.

However, mixing birch and pine did have a positive effect on mid-soil organic matter and its breakdown, which could have fueled the higher aboveground productivity found at ORPHEE. Thus, mixing tree species, even if they do not affect topsoil nutrient cycling, can have an important effect on tree growth and litterfall, increasing the quantity of litter accumulated on the forest floor. A higher litterfall flux has the potential to increase organic matter inputs if microorganism decomposition activity follows. Thus, mixing tree species may be a good solution to increase tree productivity (Belluau et al. 2021; Morin et al. 2020), although the exact mechanisms by which the species influence the soil organic matter breakdown at the mid-soil layer remain to be tested.

Finally, the choice of species studied is important to consider when designating species within a mixture (Baert et al. 2018), as these will drive aboveground growth dynamics

and canopy covering. The simple effect of mixing may not lead to synergistic interactive effects, as species identity is a stronger driver for aboveground nutrient use efficiency, at least in young plantations. The importance of species identity within a mixture has previously been put forward in studies looking into stem biomass increment in mixtures (Tobner et al. 2016), litter decomposition experiments (Jewell et al. 2017), soil microbial community respiration, biomass and composition (Khlifa et al. 2017), foliar nutrient content (Nickmans et al. 2017; Nickmans et al. 2015)) or in the sensitivity of trees to drought (Pardos et al. 2021; Vitali et al. 2018). However, if the species mixtures lead to aboveground overyielding, there may be belowground complementarity effects in deeper soil layers, as we found with an increase in organic matter and extracellular enzyme activities in the 15-30 cm soil layer. Thus, not only is the choice of planted species important, but there are potential belowground interactions which may not follow the patterns aboveground.

Limitations

In this thesis, we present new insights on nutrient cycling in mixed forests under different levels of water availability, at different spatial and temporal scales. As with all studies, there are limitations that need to be addressed. First and foremost, the stands studied were young, and the results found could change with plantation development. Indeed, tree richness effects can change over the course of decades (Sheil 2001), and stand succession leads to varying interactions between species (Widenfalk and Weslien 2009). For example, Tatsumi 2020 found that the positive impacts of tree diversity on forest biomass increased over multiple decades. The positive complementarity effect was explained differently over time: in the early stages, it was due to enhanced growth of early and mid-successional species in the mixture, while later on it was due to the higher survival rates with vertical spatial partitioning.

Secondly, while the use of plantation experiments to study biodiversity-ecosystem functioning relationships has provided us with substantial advances on theoretical grounds, there is nevertheless a lack of linkages to applied sciences and industrial practices (Verheyen et al. 2016). While the increased plantation density at IDENT-SSM allowed for more rapid species interactions, the validity of these interactions, and of the biodiversity-ecosystem functioning relationships, can be questioned when scaling up to larger applications. It would thus be pertinent to study tree mixing effects at a larger scale, i.e. similar to the 2m plantation spacing at ORPHEE, and in naturally diverse forest ecosystems.

Thirdly, we did not include the study of the understory at ORPHEE (the understory was removed at IDENT-SSM), which influences both nutrient availability and ecosystem uptake

dynamics. Understory fine roots contribute significantly to total ecosystem belowground biomass (Bakker et al. 2006; Gonzalez et al. 2013, Table S1.2). Tree species composition indirectly affected the understory biomass and community. Under pure birch, for example, there was three times more understory cover than in either the pure pine or the birch-pine mixture (Table 3.3). A previous study at our site also found that tree species composition had a significant effect on understory plant assemblage, species richness and diversity, which were in turn not affected by water availability (Corcket et al. 2020). Understory can influence several steps of nutrient cycling, such as organic matter inputs (either the aboveground *via* litterfall or belowground *via* root turnover), priming to obtain nutrients, and nutrient uptake, for example. The presence of certain understory species could also impact plot-level nutrient cycling, such as the nitrogen-fixing gorse, which was more present under birch as this shrub species needs a lot of sunlight. Indeed, the intercropping of gorse has previously been found to result in high inputs of nitrogen and organic matter into plantations of maritime pine (Augusto et al. 2005; Vidal et al. 2019). The presence of understory can also have repercussions on soil water availability, as the understory layer can lead to a moister and cooler microclimate above the soil. In a long-term experiment in Switzerland, for example, researchers found that broadleaved and non-pine conifer forests moderated daytime understory microclimate about twice as much as pine forests (von Arx et al. 2012). This could be advantageous during the summer to maintain wetter conditions during dry periods, which could lead to higher microbial activity and nutrient availability. Thus, in order to fully understand how mixing tree species may impact ecosystem-level nutrient cycling in a non-cleared forest, it is necessary to take into account the present understory.

Another point to address is the way that water availability was studied in this thesis. At ORPHEE, high water availability was controlled *via* seasonal irrigation and low water availability was the control, subject to the precipitation-dependent dry summers of south-western France. At IDENT-SSM, installed gutters assured that there would be differences in water availability between rain exclusion plots, which excluded around 25% of rainfall, and high water availability treatment *via* seasonal irrigation. While we were able to draw conclusions about the effect of differences in water availability, it would be interesting to look more precisely into the effect of severe drought and recovery, or high precipitation events, as these kinds of events are predicted to be more frequent in the future (IPCC 2014). This would help us learn more about how mixing tree species may improve ecosystem resistance and resilience to punctual disturbances.

Perspectives

Regarding the perspectives of this study, it would be interesting to investigate how water availability and tree mixing influence nutrient cycling in a natural forest, in which the growth of the different species and their take up of the canopy space stems from natural species dynamics, as opposed to standard planting in an experimental design. Indeed, we found that stand age and density could play a role on aboveground nutrient use due to differences in covering of the canopy space; similar dynamics in natural forests should be followed, since a high proportion of managed forests are natural. Similarly, it would be interesting to see whether changes in water availability influence these dynamics in natural forests. For example, the sensitivity of European beech to environmental conditions such as drought strongly depends on neighborhood identity (Metz et al. 2016).

While we were only able to study aboveground nutrient use at both sites, we found consistent conclusions, i.e. that species dominance drove this process, even though the specific species were different. It would be interesting to extend this study to the belowground processes at IDENT-SSM, but also to mixed stands with different species, climate and soil type, to investigate the potential influence of these properties. However, it would not be surprising if the conclusion would be that processes are highly context-dependent, as previously found (Adair et al. 2018; Ratcliffe et al. 2017). Nevertheless, it could help us understand the mechanisms of nutrient cycling in forests.

Some of our key findings are the differences in uptake dynamics between monocultures and mixtures due to delayed budburst in the latter, and an increase in organic matter and soil microorganism activity at the mid-soil layer. To further explore these findings, it would be interesting to look more into potential delayed budburst and nutrient uptake capacities at both budburst and leaf senescence for differing species, to assess which species may be more prone to change their phenology when mixing. The belowground spatial complementarity for mixing is also worth exploring, to look into the mechanisms behind the accumulation of organic matter in this 15-30 cm soil layer. It would also be interesting to see the temporal dynamic in this layer, as we only studied the potential temporal complementarity of mixing in the topsoil.

Finally, it would be pertinent to see how knowledge from these experimental studies can be scaled up and used in a forest management perspective. To respond to this need, the TreeDivNet group has started Application Trials, by designing new experiments with and for stakeholders and managers in connection to established TreeDivNet sites. These recent application trials are managed at an operational scale for the forestry industry and provide added value for the knowledge transfer towards forest policy and management (Messier et al. 2009). Studying nutrient cycling in these plantations would thus be interesting to

determine how mixing tree species may improve different steps of nitrogen and phosphorus cycling with the goal of producing wood.

In the present context of increasing drought frequency and severity, and forest management looking for concrete solutions for sustainable practices, I suggest the following as a priority for future experiments. (1) Testing different tree species, both native and those that could be assisted migrated (Vitt et al. 2010), to see which mixtures would be more resistant to global changes. To do so, (2) long-term experiments that manipulate both severe drought, and frequent mild droughts would be useful to understand how certain mixtures are resistant to these changes. Finally, (3) studies which look into forests as social-ecological ecosystems are needed (Fouqueray and Frascaria-Lacoste 2020), to understand the receptiveness of climate change adaptation, such as mixing tree species, by various actors (research funding bodies, forest authorities, foresters, and the general public). All together, these priorities would give us the best chance of helping our forests resist changes to the climate.

Annexes

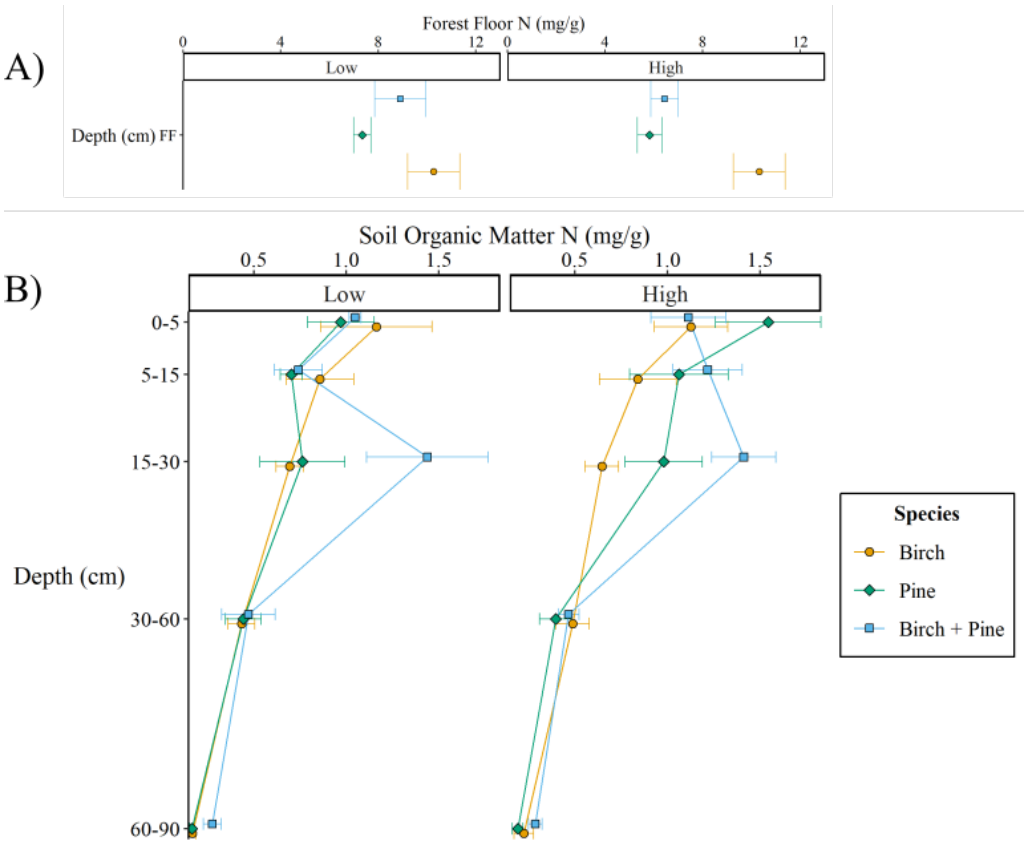

Figure E.1: Organic matter nitrogen (mg g^{-1}) in A) the forest floor stocks and B) along the soil profile. Data are presented for pure birch (*yellow*, circles), pure pine (*green*, diamonds), and mixed birch-pine (*blue*, squares) under both low and high water availability at ORPHEE. Samples were taken in March 2018.

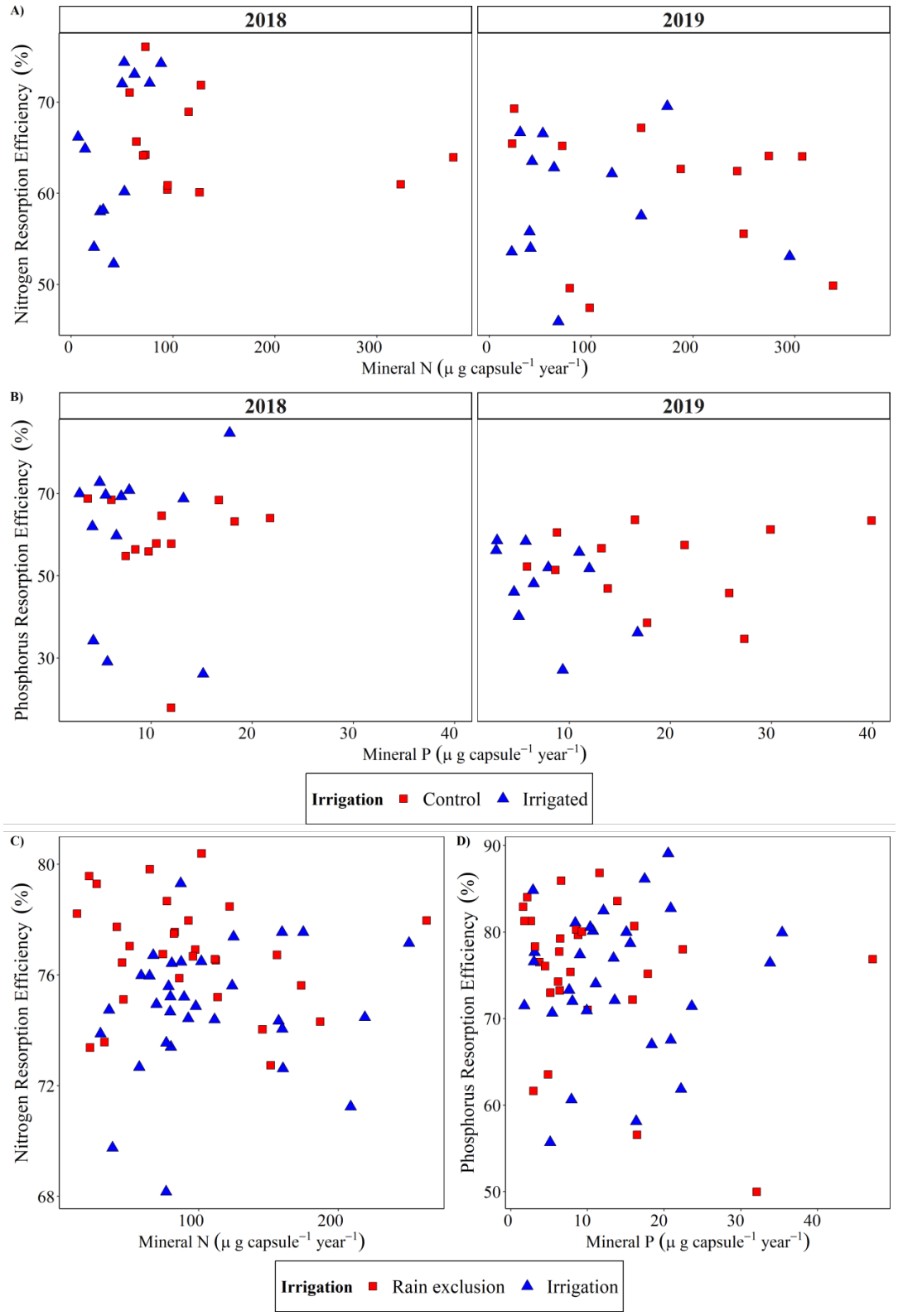

Figure E.2: Relationship between between mean soil mineral N (sum of NH_4^+ and NO_3^-) and phosphate availability and plot-level N and P resorption efficiency under two levels of water availability. Mineral N vs. nitrogen resorption efficiency at A) ORPHEE in 2018 and 2019 and C) IDENT-SSM in 2019. Phosphate vs. phosphorus resorption efficiency at B) ORPHEE in 2018 and 2019 and D) IDENT-SSM in 2019. Data points are the mean value from IER resins per plot (n = 3 resins per plot at IDENT-SSM, n = 4 per plot at ORPHEE) as there was 1 value of NutRE per plot.

Plot numbers within a block

Species studied

Pinus strobus, PIST *Betula papyrifera*, BEPA *Acer saccharum*, ACSA
Larix laricina, LALA *Quercus rubra*, QURU

Plot #	Block 1	Plot #	Block 2
1	ACSA	2	QURU
4	BEPA-PIST	4	LALA
5	QURU	5	BEPA-ACSA-LALA-PIST
8	PIST	7	BEPA
15	BEPA	12	ACSA
19	QURU-LALA	14	PIST
20	BEPA-ACSA	15	BEPA-PIST
23	QURU-ACSA-LALA-PIST	16	BEPA-ACSA
24	LALA	20	QURU-LALA
25	BEPA-ACSA-LALA-PIST	21	QURU-ACSA-LALA-PIST
26	BEPA-LALA	25	BEPA-LALA

Plot #	Block 3	Plot #	Block 4	Plot #	Block 5
2	BEPA-ACSA-LALA-PIST	2	QURU	1	PIST
3	LALA	3	BEPA-LALA	2	QURU-ACSA-LALA-PIST
7	BEPA	4	PIST	3	BEPA-PIST
9	QURU-ACSA-LALA-PIST	5	BEPA-ACSA	4	QURU
10	QURU-LALA	7	BEPA	5	BEPA-LALA
13	PIST	14	BEPA-PIST	6	BEPA-ACSA
14	BEPA-ACSA	15	LALA	7	BEPA
15	QURU	16	BEPA-ACSA-LALA-PIST	13	LALA
18	BEPA-LALA	23	QURU-LALA	18	ACSA
20	BEPA-PIST	24	ACSA	19	BEPA-ACSA-LALA-PIST
21	ACSA	26	QURU-ACSA-LALA-PIST	25	QURU-LALA

Plot #	Block 6	Plot #	Block 7	Plot #	Block 8
3	BEPA	1	QURU-ACSA-LALA-PIST	3	QURU
4	BEPA-LALA	3	BEPA-PIST	4	QURU-LALA
8	BEPA-ACSA	5	BEPA-LALA	10	BEPA-PIST
9	LALA	9	BEPA	12	LALA
10	BEPA-ACSA-LALA-PIST	10	PIST	14	BEPA
12	ACSA	11	ACSA	16	PIST
13	PIST	12	QURU	19	QURU-ACSA-LALA-PIST
14	BEPA-PIST	13	LALA	22	BEPA-ACSA
15	QURU-ACSA-LALA-PIST	14	BEPA-ACSA-LALA-PIST	23	ACSA
17	QURU-LALA	15	QURU-LALA	24	BEPA-LALA
20	QURU	26	BEPA-ACSA	26	BEPA-ACSA-LALA-PIST

Figure E.3: Detailed plot placement at the IDENT-SSM field site.

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Titre : Interaction entre la diversité des espèces d'arbres et la ressource en eau sur le recyclage des nutriments en forêt

Résumé : Augmenter la biodiversité est une solution mise en avant pour rendre les forêts plus efficientes et résistantes aux changements globaux, tels que les sécheresses. Dans cette thèse, nous avons cherché à déterminer si une disponibilité réduite en eau pouvait avoir un impact sur les effets potentiels de la diversité des espèces d'arbres sur le recyclage des nutriments. Pour y répondre, deux sites expérimentaux (France et Canada) qui manipulent à la fois la diversité des arbres et l'eau ont été étudiés. Nous avons observé que les processus biogéochimiques de la couche superficielle du sol ont été déterminés par la disponibilité de l'eau, les processus du sol intermédiaire par le mélange des espèces, et l'efficience d'utilisation de nutriments par l'identité des espèces. Enfin, nous n'avons pas observé d'effet interactif entre ces facteurs, car ils étaient séparés dans l'espace et dans le temps.

Mots-clés : biodiversité, changements globaux, cycles biogéochimiques, disponibilité des nutriments du sol, absorption des nutriments, efficience d'utilisation des nutriments

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Title: Interaction between tree diversity and water availability on nutrient cycling in forests

Abstract: Increasing biodiversity has been highlighted as a solution to make forests more efficient and resilient to global changes, such as drought. In this thesis, we sought to determine whether reduced water availability could have an impact on the potential effects of tree species diversity on nutrient cycling. To answer this, two experimental sites (France and Canada) that manipulate both tree diversity and water were studied. We observed that surface soil biogeochemical processes were driven by water availability, intermediate soil processes by species mixing, and nutrient use efficiency by species identity. Finally, we did not observe an interactive effect between these factors, as they were spatially and temporally separated.

Keywords: biodiversity, climate change, biogeochemical cycles, soil nutrient availability, nutrient uptake, nutrient use efficiency

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