

# EFFECTS OF TREE DIVERSITY, DROUGHT AND THEIR INTERACTION ON NUTRIENT CYCLING



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## Introduction

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Forests are major terrestrial ecosystems covering about 30% of worldwide land area (Food and Agriculture Organisation of the United Nations, 2015). They are particularly important for Human societies because they provide a large range of ecosystem services, such as carbon sequestration and tree biomass production (Krieger, 2001), yet they are increasingly impacted by disturbances driven by global changes such as drought. According to a landmark report on global changes (IPCC, 2018), summer dry periods are expected to be more frequent and intense in South-Western Europe, so water deficit may become one of the main constraints for plant survival and productivity. A solution to mitigate the negative effects of drought may be the incorporation of different species into forest plantations. Indeed, mixed forests have been found to stabilize tree productivity over time under varying water conditions due to a diversity of traits that can lead to species complementarity (Baeten et al., 2019; Cardinale et al., 2007; Kardol, Fanin, & Wardle, 2018), but this has been demonstrated mostly in studies focusing on the aboveground biomass. Little is known about how diverse forests can influence belowground biomass and soil functioning, especially when considering global changes. In particular, the role of tree diversity and its interaction with water availability on root systems and microbial functioning is still poorly understood.

Soil enzyme activities (SEA) are important for carbon (C) and nutrient (nitrogen (N) and phosphorus (P)) cycling because they act on chemical bonds within molecules during the decomposition and mineralization of soil organic matter (SOM) (Alberti et al., 2017; Allison, Gartner, Holland, Weintraub, & Sinsabaugh, 2007; Burns et al., 2013; Sinsabaugh et al., 2008). Released by microorganisms, they are useful proxies to assess microbial limitations in soils because they catalyse the first steps of catabolism by converting complex organic compounds into smaller dissolved compounds (Allison et al., 2007). This allows the assessment of how much energy microbial communities invest to acquire particular nutrients from SOM (Fatemi, Fernandez, Simon, & Dail, 2016). For instance, the C-related enzymes  $\beta$ -1,4-glucosidase,  $\beta$ -D-cellobiosidase,  $\alpha$ -1,4-glucosidase, and  $\beta$ -xylosidase, the N-related enzymes  $\beta$ -1,4-N-acetylglucosaminidase, L-leucine aminopeptidase and the P-related enzyme acid phosphatase are good proxies of C, N and P cycling, respectively (Sinsabaugh et al., 2008). Consequently, C-, N- and P-related enzymes are commonly used to measure microbial demand in energy and nutrients (Dong, Wang, Liu, Xu, & Zeng, 2019; Fanin, Moorhead, & Bertrand, 2016; Rosinger, Rousk, & Sandén, 2019), although they are also influenced by several abiotic factors such as soil pH, moisture and temperature (Kreyling et al., 2008; Pei et al., 2016; Steinauer et al., 2015). Several studies showed that depth has a negative effect on SEA (Hsiao, Sassenrath, Zeglin,

Hettiarachchi, & Rice, 2018), likely because nutrient supply or substrate quality varies along the soil profile (Loeppmann, Blagodatskaya, Pausch, & Kuzyakov, 2016), or because microbial biomass decreases with soil depth (Stone, DeForest, & Plante, 2014).

Over the last decades, a growing number of papers have focused on either the effect of drought or of tree diversity on SEA, but not on their interaction. Although the majority of papers demonstrated that drought significantly decreased SEA (Brockett, Prescott, & Grayston, 2012; Geisseler, Horwath, & Scow, 2011; Li & Sarah, 2003; Sardans & Peñuelas, 2005), some other did not find any influence of water availability on SEA (Kreyling et al., 2008; Sardans, Peñuelas, & Estiarte, 2008). SEA may also depend on tree species because varying litter quality and quantity is known to alter decomposition rates, such as *via* an effect on the decomposer communities (Chapman, Newman, Hart, Schweitzer, & Koch, 2013; Cornwell et al., 2008). Furthermore, mixed plantations may present various forms of species complementarity through a better niche partitioning in space and time (Brassard et al., 2013; Cardinale et al., 2007) or a plasticity in uptake of different forms of nutrients (Miller, Bowman, & Suding, 2007), resulting in reduced competition between co-occurring plants (Loreau & Hector, 2001). However, although a synergistic relationship between SEA and plant biodiversity has been observed across various ecosystems (Alberti et al., 2017; Kreyling et al., 2008; Steinauer et al., 2015), the interaction of tree diversity and drought on microbial limitations and its impact on nutrient availability has so far never been evaluated experimentally in the field.

For a more mechanistic understanding of the interactive effect of drought and tree diversity on soil functioning, our main objective was to investigate the regulatory mechanisms that control SEA. To do so, we sampled soils in a 10-year-old experimental platform in Aquitaine (France) which manipulates both tree species richness and water resource by irrigation. We collected soil samples at five different depths (0-5, 5-15, 15-30, 30-60 and 60-90 cm), analysed SEA, and compared results to previously acquired data on bioavailable N and P, and total root length and biomass. The inclusion of depth in our study allows us to investigate whether the effects of drought, tree diversity and their interaction vary along the soil profile. Because most studies have found that drought has a negative effect on soil nutrient cycling, we hypothesized that (i) drought would have a negative effect on SEA ( $H_1$ ). Similarly, because several studies have reported that biodiversity has a positive influence on soil functioning, we hypothesized that (ii) tree diversity would have a positive effect on SEA ( $H_2$ ). Finally, because the effects of drought may differ among studies (Brockett et al., 2012; Li & Sarah, 2003), we propose that these effects are modulated by tree diversity due to complementarity effects (Brassard et al., 2013; Ratcliffe et al., 2017). Accordingly, we stipulated that (iii) tree diversity

would mitigate the negative effect of drought on SEA ( $H_3$ ). Overall, by disentangling the relative importance of drought and tree diversity and their interaction on SEA, this study aims to improve our understanding of the context-dependency of the effects of biotic and abiotic factors on soil microorganism activity in temperate forests.

## Material & Methods

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### *Study site*

The ORPHEE experiment is located 40 km south-west of Bordeaux (France) in the Landes de Gascogne forest. It was established in January 2008 on 12 ha of a maritime pine clear cut field and the soil is composed of 95% sand, 2% loam and 3% clay (Table S1), with an acidic pH around 4.5. The site is organised into 8 blocks, each one being composed of 32 plots. Combinations of 1, 2, 3, 4 and 5 species have been planted in each block. In this study, we will focus specifically on three of these plots including monocultures of silver birch (*Betula pendula*) and maritime pine (*Pinus pinaster*) and the combination of the two. The plots are 20 m by 20 m and randomly distributed within each block. The trees were planted 2 m apart, so there is a total of 100 trees per plot. An irrigation system was set up in 2014 in the plots of four out of the eight blocks. A sprinkler located at the centre of each plot sprays a quantity of water supply (3 mm/night/plot) that has been calculated to compensate for water stress during the drier months of the year in the Landes de Gascogne, from May to October. This allows us to assess the effect of water availability in interaction with tree diversity on several belowground variables: soil enzyme activities, available nutrients and root traits.

### *Soil collection*

Four soil cores were sampled in each plot in 2018 from March 19<sup>th</sup> to 21<sup>st</sup>. The cores were collected between four representative trees of the respective plot to 1 m depth using a driller. The soil corer was 15 cm in depth with a diameter of 8 cm for the upper soil layers, and of 4 cm for the lower soil layers. Each soil core was divided into five horizons: 0-5 cm, 5-15 cm, 15-30 cm, 30-60 cm and 60-90 cm. In total, 480 samples were collected in the field (5 depths  $\times$  4 cores  $\times$  3 plots  $\times$  4 blocks  $\times$  2 water conditions). In the lab, each sample was sieved using a 2 mm sieve in order to remove stones and roots. A soil subsample was kept fresh at 4°C for available N and P analyses. The roots were cleaned and stored in 10% alcohol until further analyses. Another soil subsample was frozen at -20°C until the enzyme assays.

### ***N and P analyses***

Available N and P were extracted from fresh soil samples using ultrapure water and analysed by colorimetry (San++, Automated Wet Chemistry Analyzer, Breda, Netherlands). In brief, 15 mL of ultrapure water were added to a glass vial with 3 g of the soil sample, which was then shaken for 16 h at 4°C. The solution was placed vertically at ambient temperature for an hour to allow sedimentation to occur, after which the supernatant was transferred into a tube through a Whatman filter of 8 µm 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 extractant was poured into a scintillation tube to measure  $\text{NO}_3^-$ ,  $\text{NH}_4^+$  and  $\text{PO}_4^-$  by colorimetry. The total available mineral N was expressed as the sum of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  (mg N g<sup>-1</sup> dry soil) and available mineral P as  $\text{PO}_4^-$  (mg P g<sup>-1</sup> dry soil).

### ***Root analyses***

For each sample, the root biomass and length were measured for all species. Root extraction consisted in sieving the collected soil samples and then watering the remaining roots to clean them and remove the soil. The live roots were sorted and the different species were separated from one another before weighing each of them. A representative portion of each root sample was placed in a tray and spread to avoid overlap, and then scanned using WinRHIZO (García-Palacios, Prieto, Ourcival, & Hättenschwiler, 2016). This last step can provide a multitude of different data, but we only used the total root length for each species.

### ***Enzyme assays***

We measured the potential activity of seven hydrolytic soil enzymes that degrade organic C [ $\beta$ -1,4-glucosidase (BGLU), 1,4- $\beta$ -D-cellobiohydrolase (CBH),  $\alpha$ -1,4-glucosidase (AGLU), and  $\beta$ -xylosidase (XYL)], N [ $\beta$ -1,4-N-acetyl-glucosaminidase (NAG), L-leucine aminopeptidase (LAP)], and P [acid phosphatase (AP)] (Bell et al., 2013; Fanin et al., 2016). The assays were conducted by homogenizing 2.75 g of the soil sample in 91 ml of 50 mM sodium acetate buffer (enzyme activities were measured at soil pH) in a blender for 1 min. Soil slurries 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  $\mu$ l of supernatant was transferred from each well into a black flat-bottomed 96-well plate and then scanned on a microplate reader (Synergy H1 microplate reader, Biotek, Winooski, USA) using an excitation wavelength at 365 nm and emission wavelength at 450 nm. Soil moisture was assessed for each sample by comparing the fresh soil weight to the dry soil weighted after 72 hours of incubation at 50°C. Enzyme activities were calculated as rates in  $\text{nmol g}^{-1} \text{ dry soil h}^{-1}$ .

### ***Data analyses***

All statistical analyses were performed using the RStudio version 3.5.2 statistical software for Windows (The R Foundation for Statistical Computing 2018). Enzyme activities were assessed both separately and in combination for each element: C [BGLU + CBH + XYL + AGLU], N [NAG + AP] and P [AP]. Enzyme activities, available nutrients, and root traits were visualized using boxplots to assess the shifts in enzyme activities among the different treatments and depths; extreme values were removed following the outlier labelling rule with a conservative tuning parameter of  $g = 2.2$  (Hoaglin & Iglewicz, 1987). The boxplots characterize the lower quartile, median, upper quartile and interquartile range (upper quartile – lower quartile), which covers the central 50% of the data; the whiskers represent 95% of the data. Finally, we divided the C-related enzyme activities by the N-related enzyme activities to determine the effects of tree diversity, irrigation, and depth on the ratio of C:N enzyme activities. We similarly calculated the C:P and N:P enzyme activity ratios to study the eco-enzymatic stoichiometry.

We then used mixed models to assess the effects of tree biodiversity treatments (pure pine vs. pure birch vs. mixed species stand), water availability (control vs. irrigation), soil depth and their interaction on SEA. Block was included as a random effect 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. We then applied contrasts and performed post-hoc tests (Tukey's HSD test,  $\alpha = 0.05$ ) to assess significant differences in C:N and C:P enzyme ratios among treatments. Then, a redundancy analysis (RDA) was done to determine the relationship between the different individual enzymes, bioavailable nutrients, soil moisture, and fine root traits. These last three were included as environmental variables. In the RDA, the ordination axes were constrained to be linear combinations of the seven individual enzymes and environmental variables. Supplementary variables representing enzyme activity ratios were then added to the RDA.

## Results

### *C-, N- and P-related enzyme responses to irrigation, diversity and depth*

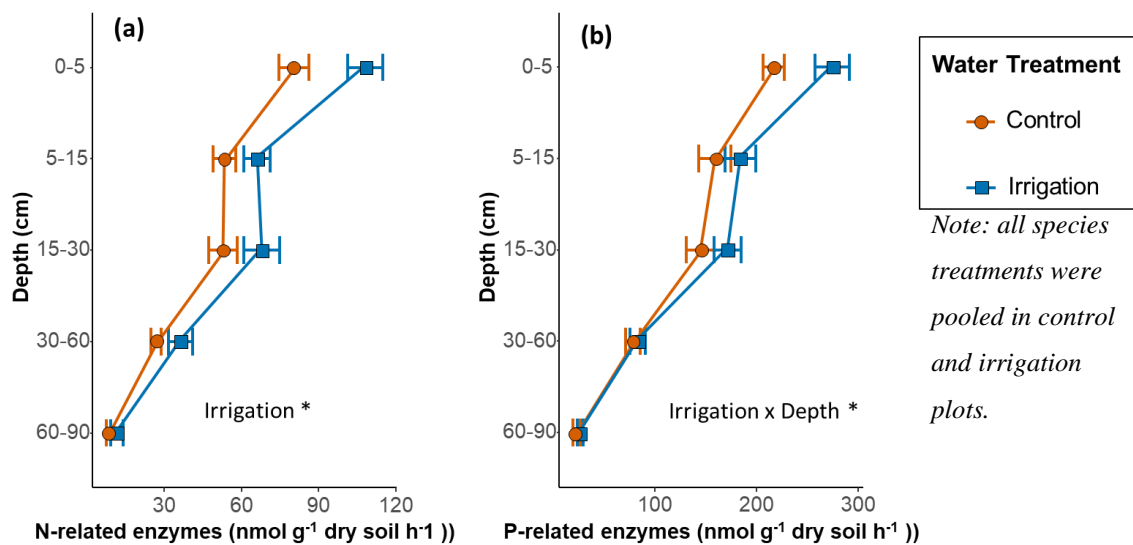
After 5 years of irrigation and 11 years of plantation on the study site, we found that the responses to the tree species and water availability treatments differed from an enzyme to another in our soil system (Table 1; Table S2; Figure S1). Our results showed an overall positive

**Table 1** Results of the linear mixed effects models to test for the effects of species, irrigation, depth and their interactions on C-, N- and P-related SEA.

Variables	Carbon related enzymes				Nitrogen related enzymes				Phosphorus related enzyme			
	numDF	denDF	F-value	p-value	numDF	denDF	F-value	p-value	numDF	denDF	F-value	p-value
<b>Species (S)</b>	2	12	2.87	<i>NS</i>	2	12	0.95	<i>NS</i>	2	12	0.26	<i>NS</i>
<b>Irrigation (I)</b>	1	6	4.89	<i>NS</i>	1	6	6.91	<b>0.0392</b>	1	6	1.38	<i>NS</i>
<b>Depth (D)</b>	4	350	159.74	<b>&lt;.0001</b>	4	349	103.86	<b>&lt;.0001</b>	4	351	155.38	<b>&lt;.0001</b>
<b>S × I</b>	2	12	0.82	<i>NS</i>	2	12	10.03	<i>NS</i>	2	12	0.10	<i>NS</i>
<b>S × D</b>	8	350	7.20	<b>&lt;.0001</b>	8	349	2.45	<b>0.0138</b>	8	351	2.31	<b>0.0199</b>
<b>I × D</b>	4	350	1.88	<i>NS</i>	4	349	1.77	<i>NS</i>	4	351	2.51	<b>0.0418</b>
<b>S × I × D</b>	8	350	0.58	<i>NS</i>	8	349	0.43	<i>NS</i>	8	351	0.20	<i>NS</i>

Note: soil moisture was used as covariable in the model. Significant results are in bold font; *NS* stands for “not significant”.

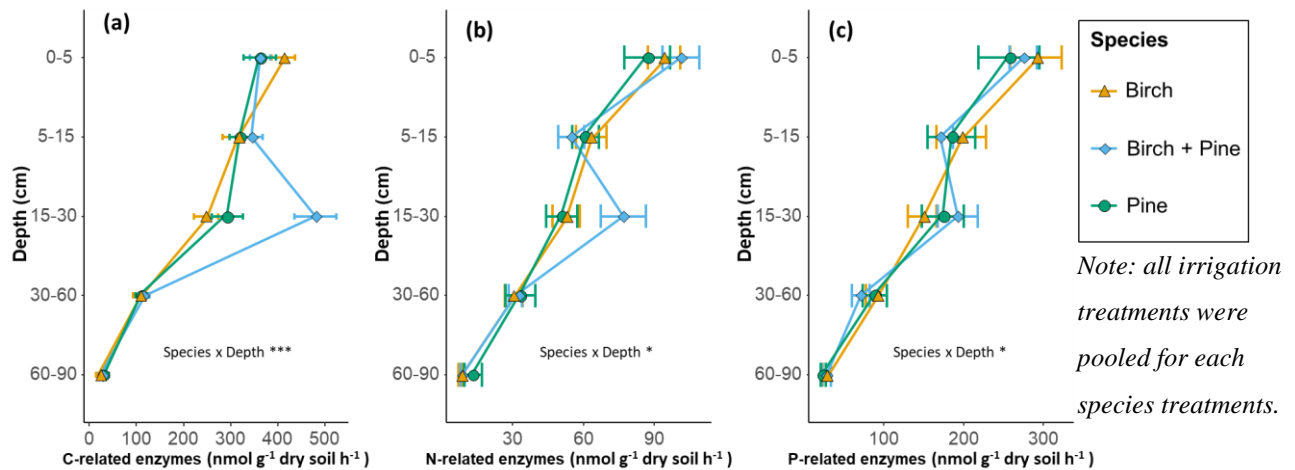
effect of irrigation on N-related enzymes (Table 1, Figure 1a): looking at averaged results of all horizons, N-related activity rates increased from 44.13 ( $\pm$  37.72) in non-irrigated blocks to 57.98 ( $\pm$  49.40) nmol g<sup>-1</sup> h<sup>-1</sup> in irrigated blocks (Table S2). In contrast, irrigation did not affect C-related enzymes. The significant effect of irrigation on P-related enzymes depended on soil depth (Table 1): although there was no significant difference per horizon, SEA tended to be higher only in the 0-5 cm horizon in control plots compared with irrigation plots (Figure 1b). For the species treatments, we found an interactive effect between tree diversity and soil depth



**Figure 1** N-related enzyme activities (a) and P-related enzyme activities (b) along the soil profile in both irrigated (blue) and control (red) blocks. The three species treatments were pooled for each water treatment.



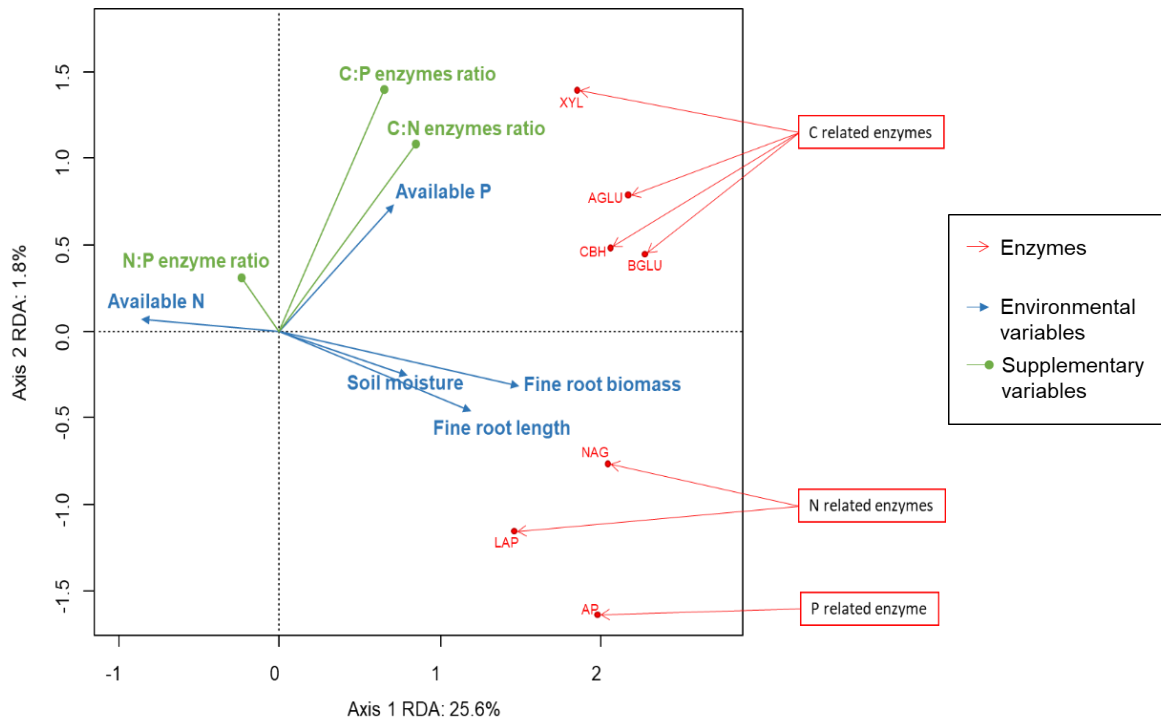
for the three SEA (Table 1, Figure 2). However, these effects depended on the enzyme considered (Figure 2). We performed Tukey post-hoc tests per horizon to determine if the treatment was significant at certain depths, and found a significant difference at 15-30 cm for C-related enzymes (Figure 2a), with the activities in the mixed-species stands being significantly higher than the activities in both pine ( $p = 0.00112$ ) and birch monocultures ( $p = 0.0001$ ). Although we did not find any significant difference per horizon, we observed a similar trend for N- and P-related enzymes (Figure 2b and 2c).



**Figure 2** C- (a), N- (b) and P-related enzymes (c) depending on the species treatment along the soil profile.

### *Relationships between enzymes and environmental variables*

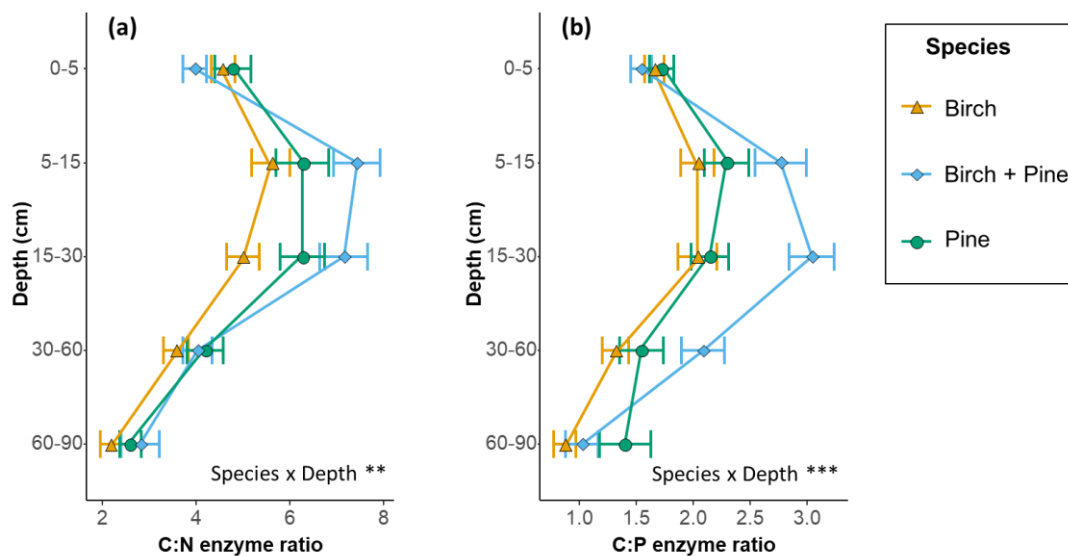
In the RDA, we found that the enzymes were segregated along the second axis depending on the element considered (Figure 3): C-related enzymes were related to one another in the top right corner at the opposite of N- and the P-related enzymes at the bottom right corner. Available N was negatively related to N-related enzymes along the first axis, and, to a lesser extent, a similar negative relation between available P and P-related enzyme was observed along the second axis (Figure 3). Root length, root biomass, and soil moisture were strongly separated from the available N by the first axis. Available P fits closely with the C- related enzymes, which explains why the C:P and C:N enzyme ratios (supplementary variables in green, Fig. 3) were closely related to the available P.



**Figure 3** Redundancy Analysis (RDA) which gives insight into how the individual SEA (red) are influenced by the environmental variables (blue). The supplementary variables (green) were added after the analysis.

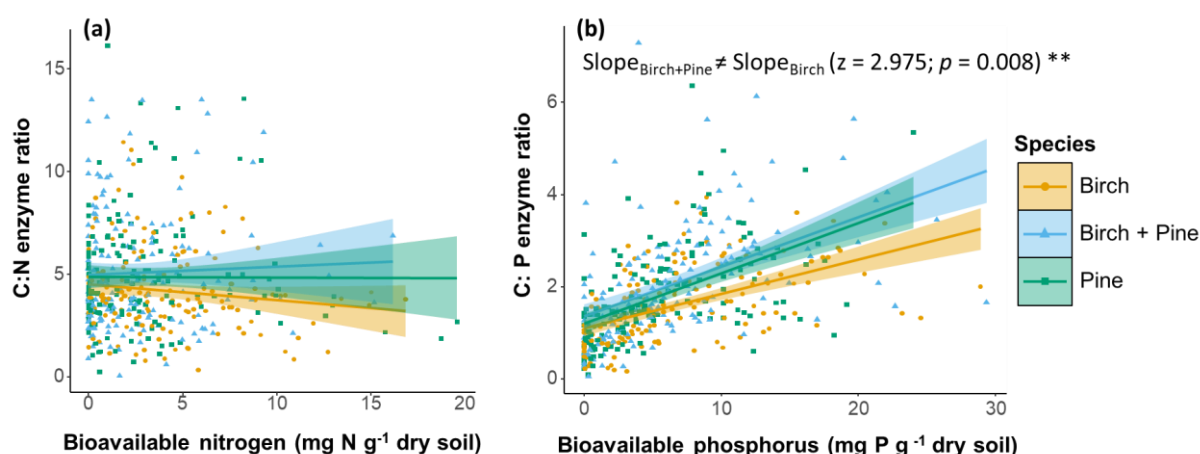
### *Eco-enzymatic stoichiometry and nutrient availability*

We found significant linear relationships between C- and N- related enzymes ( $r^2 = 0.82$ ,  $p < 0.0001$ ), C- and P-related enzymes ( $r^2 = 0.80$ ,  $p < 0.0001$ ) and N- and P- related enzymes ( $r^2 = 0.77$ ,  $p < 0.0001$ ) (Figure S2). When investigating enzyme stoichiometry, we found that the effect of the species treatments on the C:N and C:P enzyme ratios depended on soil depth



**Figure 4** C:N (a) and C:P (b) enzyme ratios depending on the species treatment along the soil profile.

(Figure 4). The C:N enzyme ratio was significantly higher in the mixture treatment than in the birch treatment at 15-30 cm ( $p < 0.00159$ ), but there was no significant difference between treatments in the other soil horizons (Figure 4a). The C:P enzyme ratio was significantly higher in the mixed species plot than in the birch plot in 2 out of 5 horizons (5-15 cm horizon:  $p = 0.0356$  and 30-60 cm horizon:  $p = 0.00858$ ) and higher than both monocultures in the horizon 15-30 cm (both  $p < 0.001$ ), whereas there was no significant difference in the 0-5 and 60-90 cm horizons (Figure 4). When using all data across the different soil depths, we did not find any relationship between the C:N enzyme ratio and available N (Figure 5a). However, we found a positive significant correlation between the C:P enzyme ratio and the available P, but this relationship varied between the species treatments (Figure 5b). Using a contrasts matrix and Tukey post-hoc tests, we found that the slope for the mixed species stand was significantly higher than that for the pure birch stand, while the slope for the pure pine stand is an intermediate between the other two (Figure 5b).



**Figure 5** Relationship between C:N enzyme ratio and bioavailable N (a), and between C: P enzyme ratio and bioavailable P (b) for the three species treatments.

## Discussion

By using a long-term irrigation and diversity manipulation experiment set up in the Landes de Gascogne, we investigated the effects of drought, tree diversity and their interaction on SEA. We focused on three hypotheses: (i) drought would have a negative effect on SEA, (ii) tree diversity would have a positive effect on SEA, and (iii) biodiversity would mitigate the negative effect of drought on SEA because of an interaction between tree diversity and irrigation treatments. Our results are in agreement with our first and second hypotheses, thereby demonstrating that SEA directly depend on tree species and water conditions in temperate forests. However, in contrast to our last hypothesis, we did not find an effect of the interaction

of drought and diversity on SEA, underlying that the negative effect of drought cannot be upset by the positive effect of biodiversity.

### ***Effect of drought***

In agreement with our first hypothesis, we found a negative effect of drought on N-related enzyme activities (Figure 1). This is probably due to a decrease in both microbial biomass (Brockett et al., 2012) and root biomass (Brunner, Arend, Dawes, Sperisen, & Herzog, 2015) under suboptimal water conditions, which may, in turn, decrease enzyme production in drought treatments. This hypothesis is supported by our RDA results, where root biomass and root length point in the same direction as soil moisture (Figure 3), demonstrating a positive link between these variables. Alternatively, Geisseler et al. (2011) showed that soil drying restricts the diffusion of extracellular enzymes away from microorganisms, which may have negatively impacted N-related enzymes in the non-irrigated control plots. However, this hypothesis is not supported by the fact that C-related enzymes were not affected by drought in our experiment (Table 1). Instead, this suggests that changes in organic matter quality are the main driver of SEA under drought, a claim that is supported by C:N ratios tending to be lower in controls plots compared with irrigation plots (Table S1). Indeed, higher tree growth rates when water conditions are favourable reduce available N in soils, with further consequences on N limitations in our study system. In line with the results on N-related enzyme activities, drought negatively affected P-related enzymes, but this effect interacted with soil depth (Table 1, Figure 1). This is likely because phosphate ions ( $\text{PO}_4^{3-}$ ) can be adsorbed to soils making them very immobile, thereby affecting P-related enzymes only in the top soil (Johnson & Cole, 1980). Taken together, these results highlight that climate changes may have important consequences on SEA through direct effects of water availability and indirect effects of trees on organic matter quality.

### ***Effect of tree biodiversity***

According to our second hypothesis, we found that the mixture treatment had a positive effect on C-, N- and P-related enzymes in interaction with soil depth (Table 1, Figure 2). For C-related enzymes, this effect was only significant at the 15-30 cm horizon (Figure 2). This is likely because roots are more present in this soil horizon as a result of a greater niche partitioning in root systems when birch and pine co-occur in the mixed-species plots (data not shown). A higher root biomass in turn may increase tree nutrient uptake and microorganism activity, thereby increasing SEA. This is in agreement with Brassard et al. (2013), who provided some

evidences of belowground species complementarity in space, resulting in an increase in nutrient uptake in mixed forests. Furthermore, changes in tree species diversity may lead to a shift in the structure of microbial communities (Pei et al., 2016). For instance, changes in the fungi to bacteria ratios may alter the stoichiometric requirements at the community scale (Fanin, Fromin, Buatois, & Hättenschwiler, 2013), which could explain the reduced effect of tree diversity on N- and P-related enzymes compared to C-related enzymes.

Changes in SEA also significantly affected C:N and C:P enzyme ratios (Figure 3). When looking at the correlation between nutrients and enzyme ratios, we found a positive relationship between bioavailable P and the C:P enzyme ratio across all treatments. As the available P increases, microorganisms release more C-related enzymes. This is probably because P limits the microbial activity in these P-poor podzol soils (Achat et al., 2009; Augusto, Achat, Jonard, Vidal, & Ringeval, 2017). However, we found that the slope of this relationship was higher for mixed plots compared with birch monocultures. This may indicate that the mixed-species plots are less limited by P than the birch plots, likely due to an increase in quantity and quality of organic matter returning to the soil (Chapman et al., 2013; Handa et al., 2014). Alternatively, changes in microbial community structure in mixed-species plots may generate different needs and requirements with further consequences on C and P cycling (Fanin et al., 2016; Güsewell & Gessner, 2009).

### ***Effect of the drought × biodiversity interaction***

In contrast to our third hypothesis, the interaction between drought and tree diversity did not affect SEA (Table 1). This is surprising given that many studies have shown that biodiversity may become more beneficial as water limitations increase (Ratcliffe et al., 2017). However, this may be because the importance of tree diversity is often context-dependent, as was shown in several other studies (Fanin et al., 2018; Grossiord et al., 2014; Liang et al., 2016; Pretzsch, Schütze, & Uhl, 2013). Moreover, we collected our samples in march, when there is sufficient precipitation and the differences in soil water content are the lowest. This may significantly reduce the impact of irrigation and the role of biodiversity in control plots. Although many studies state that biodiversity can mitigate the effect of drought (Lebourgeois, Gomez, Pinto, & Mérian, 2013; Pretzsch et al., 2013), the results of our experiment suggest that the negative effect of drought on SEA cannot be upset by the positive effect of tree diversity. Supplementary analyses including the 3-species plots with oak (soil samples available), and more sampling dates are needed to further evaluate the relationship between SEA and diversity, as well as potential temporal changes of SEA.

### ***C-, N- and P-related enzyme responses to environmental variables***

Available N appeared to be negatively correlated with root traits (Figure 3), likely due to rapid nutrient absorption by roots, which leads to N-depletion where roots thrive (Giehl & Wirén, 2014). N-related enzymes were correlated to root traits probably due to an increase of soil microbial activity stimulated by root exudation (Renella, Egamberdiyeva, Landi, Mench, & Nannipieri, 2006; Zhang, Dippold, Kuzyakov, & Razavi, 2019). Indeed, when the supply in bioavailable N is low, microorganisms tend to release N-related enzymes to maintain high growth rates (Kaye & Hart, 1997). To a lesser extent, P-related enzymes were negatively correlated to available P, likely due to a similar feedback. However, we also found that available P was correlated to the C-related enzymes meaning that when P availability is high, microorganisms are strongly limited by C, inciting them to produce more C-related enzymes to get the access to the energy from the decomposing organic matter. In agreement with Achat et al. (2009) and Trichet et al. (2009), our results reinforce the idea that P is the main limiting nutrient in the Landes de Gascogne.

### **Conclusions**

The work that we describe in this experiment reveals that drought and biodiversity may have important long-term effects on soil enzyme activities (SEA), especially when considering the expected increase in water deficits in the upcoming years. Our results are in partial agreement with our first hypothesis about drought negatively affecting SEA (negative impacts on N- and P- but neutral for C-related enzymes), and in agreement with our second hypothesis suggesting that biodiversity has a positive effect on SEA. However, in contrast to our third hypothesis, biodiversity effects did not mitigate the negative impact of drought on SEA. These findings have important implications for the decomposer subsystem and therefore for fluxes of C, N and P belowground. First, our results highlight that changes in climatic conditions and tree diversity may have important consequences on SEA through direct effects of water availability and indirect effects on organic matter quality. Second, from a management perspective, they suggest that biodiversity may become of greater importance because it helped reduce the limitations in phosphorus and increased SEA, thereby accelerating nutrient cycling. However, increasing tree diversity is unlikely to mitigate the negative effects of climate change on soil functioning, although the biodiversity effects may vary with time (Cardinale et al., 2012). The next step will be to sample at different seasons to investigate the potential temporal variations of SEA to assess the dynamics in the interactive effects between drought and tree diversity over time.

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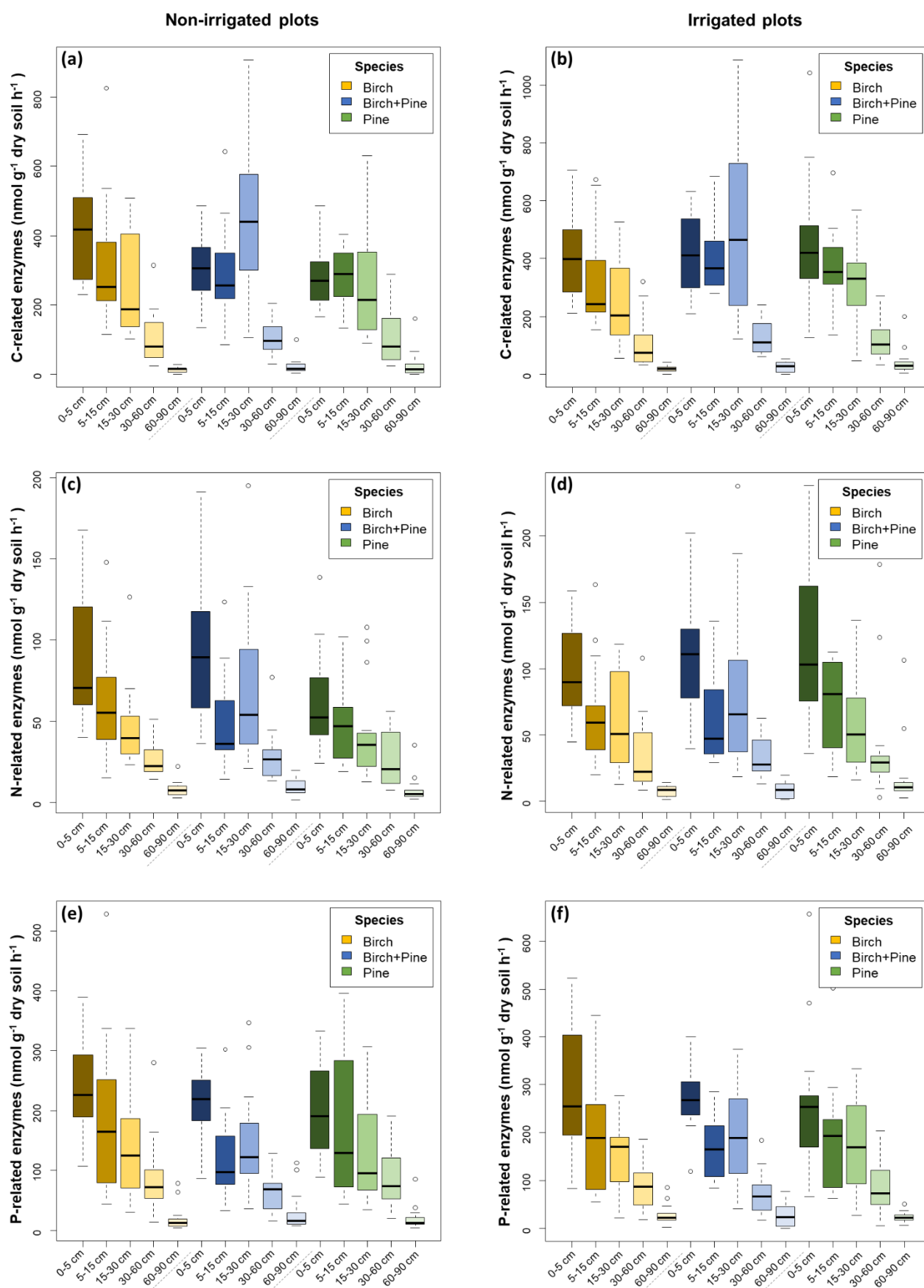
## Supplementary material

**Table S1** Supplementary environmental variables in irrigated vs. non-irrigated plots for all horizons. All species treatments have been pooled.

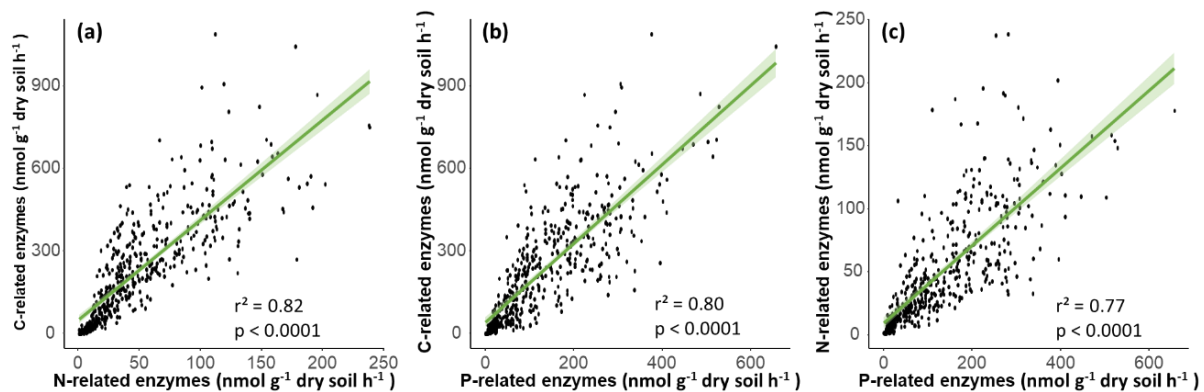
Irrigation	Depth									
	0-5 cm		5-15 cm		15-30 cm		30-60 cm		60-90 cm	
	NO	YES	NO	YES	NO	YES	NO	YES	NO	YES
Sand (g/kg)	936.50 ± 8.54	934.50 ± 7.85	943.00 ± 8.76	923.25 ± 12.89	938.50 ± 6.03	919.50 ± 21.08	974.25 ± 1.26	973.00 ± 3.56	979.25 ± 12.89	982.25 ± 2.75
Loam (g/kg)	25.00 ± 4.97	29.25 ± 6.75	24.75 ± 4.03	38.00 ± 11.89	24.75 ± 5.32	36.25 ± 7.80	6.00 ± 2.00	8.75 ± 3.86	6.00 ± 6.73	3.75 ± 1.26
Clay (g/kg)	38.50 ± 4.04	36.25 ± 2.50	32.25 ± 5.12	38.75 ± 6.70	36.75 ± 4.92	44.25 ± 13.57	19.75 ± 0.96	18.25 ± 1.89	14.75 ± 6.18	14.00 ± 1.83
Organic C (mg/g)	31.49 ± 5.83	41.06 ± 10.47	24.66 ± 5.22	36.69 ± 9.57	30.72 ± 6.22	34.84 ± 3.72	14.72 ± 6.06	14.45 ± 3.42	6.93 ± 1.07	7.89 ± 2.59
Total N (mg/g)	1.06 ± 0.26	1.26 ± 0.28	0.77 ± 0.14	1.04 ± 0.22	0.96 ± 0.18	1.01 ± 0.12	0.45 ± 0.13	0.45 ± 0.11	0.20 ± 0.03	0.23 ± 0.07
C:N	30.25 ± 2.99	32.57 ± 2.47	32.33 ± 3.52	35.01 ± 2.44	32.59 ± 2.93	34.58 ± 2.30	32.12 ± 3.34	32.04 ± 2.55	34.85 ± 4.40	33.80 ± 2.16

**Table S2** Means (and standard deviations) of C-, N- and P-related enzyme activities for the species treatments (irrigation treatments pooled) and for the irrigation treatments (species treatments pooled).

	Cenzym	Nenzym	Penzym
<b>Birch (BP)</b>	219.12 ± 188.36	49.38 ± 41.19	142.24 ± 119.63
<b>Pine (PP)</b>	224.06 ± 186.33	48.99 ± 44.36	134.5 ± 36.00
<b>BP × PP</b>	265.31 ± 220.24	54.57 ± 47.50	130.81 ± 99.29
<b>No irrigation</b>	217.15 ± 183.97	44.13 ± 37.72	124.33 ± 102.00
<b>Irrigation</b>	255.81 ± 213.20	57.98 ± 49.40	147.97 ± 120.29



**Figure S1 C-** (a,b), N- (c,d) and P-related enzymes activities (e,f) in control plots (a,c,e) and irrigated plots (b,d,f) for all species treatments along the soil profile.



**Figure S2** Correlations between N- and C-related enzymes ( $n = 235$ ) (a), P- and C-related enzymes ( $n = 239$ ) (b), and P- and N-related enzymes ( $n = 236$ ) (c). Statistical significance comes from Pearson's product-moment correlation test.

## Contributions

This internship is a part of the BEnEFIC project (BiodivErsité, Ecosystèmes ForestIers et Climat) coordinated by Nicolas Fanin (ISPA) and of the DiPTiCC project (Diversity and Productivity of Trees in the context of Climate Change), an ANR (Agence Nationale de la Recherche) project coordinated by Hervé Jactel (Biogeco). The hypotheses were formulated by Lucie Bon (ISPA), with the help and validation of Nicolas Fanin and Tania Maxwell (ISPA). The experimental design used during this internship was developed by Nicolas Fanin. Soil collection was done in March 2018 by Nicolas Fanin, Laurent Augusto, Mark Bakker Sylvie Milin, Catherine Lambrot, Andreas Altinmazis Kondylis and Kathina Mussig (all members of ISPA). Nutrient extractions were realized by Adèle Courbineau, Sylvie Milin and Coralie Chesseron at the INRA of Villenave d'Ornon and nutrient concentrations were measured by Catherine Lambrot at the INRA of Cestas-Pierroton. Root traits were measured by Andreas Altinmazis Kondylis, Kathina Müssig and Mark Bakker at the INRA of Villenave d'Ornon. The enzyme activities measurements were realized at the INRA of Villenave d'Ornon (Bordeaux) by Lucie Bon, who was trained by Sylvie Milin (ISPA), and occasionally assisted by Nathalie Gallegos (ISPA). Data formatting was done by Lucie Bon, according to Tania Maxwell's organisation. Tania Maxwell provided a script as a base, that was adapted by Lucie Bon to perform the statistical analyses. Lucie Bon analysed the data, choose the results and drew the figures in close consultation with Nicolas Fanin. Lucie Bon wrote the first draft of the manuscript with the help of Tania Maxwell and Nicolas Fanin. In addition to this project, Lucie Bon participated in field sampling for other projects in Aquitaine and in the Alps (see planning below) with Mark Bakker, Laura Scolan (ISPA) and Nicolas Fanin.

## **Planning of Lucie Bon's internship, starting 07/01/2019 and ending 28/06/2019:**

January		February		March		April		May		June	
1	M			1	V	Solutions		1	M		
2	M			2	S			2	J	Field prep	
3	J			3	D			3	V	Field prep	
4	V			4	L	Enzymes		4	S		
5	S			5	M	Enzymes		5	D		
6	D			6	M	Enzymes		6	L		
7	L	Start		7	J	Enzymes		7	M		Report
8	M			8	V	Enzymes		8	M		
9	M			9	S			9	J		
10	J	Solutions		10	D			10	V		
11	V	Solutions		11	L			11	S		
12	S			12	M			12	D		
13	D			13	M			13	L		
14	L	Enzymes		14	J	Orals		14	M		
15	M	Abstract		15	V			15	M		
16	M	Enzymes		16	S			16	J		
17	J	Enzymes		17	D			17	V		
18	V	Enzymes		18	L	Enzymes		18	S		
19	S			19	M	Enzymes		19	D		
20	D			20	M			20	L		Orals
21	L	Enzymes		21	J	Solutions		21	M		Orals
22	M	Enzymes		22	V	Solutions		22	M		
23	M	Enzymes		23	S			23	J		
24	J	Enzymes		24	D			24	V		
25	V	Enzymes		25	L	Enzymes		25	S		
26	S			26	M	Enzymes		26	D		
27	D			27	M	Enzymes		27	L		
28	L			28	J	Enzymes		28	M		End
29	M			29	V	Enzymes		29	M		
30	M			30	S			30	J		
31	J	Solutions		31	D			31	V		

	Lab
	Field work
	Desk work
	Days off
	University work

## **Acknowledgements**

I thank my two supervisors, Tania Maxwell and Nicolas Fanin, for their advice, their help, their availability and making this internship possible. It was always possible to have great conversations and to laugh with you, especially on the field. I would also like to thank Sylvie Milin and Nathalie Gallegos for their help and advice in the lab, but mostly for their great company during the whole experience. A big thanks to all of the other trainees and PhD students for always being excited about lunch and coffee breaks, and beers. A special dedication to Laura Scolas, my partner in crime in the Alps and at the INRA. I also thank all of the ISPA team for their welcome during this internship, particularly Mark Bakker and Laurent Augusto who helped me with my presentations and were invested in my internship.

## Abstract

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Forests are major terrestrial ecosystems providing a large range of ecosystem services, yet they are increasingly impacted by droughts amplified by global changes. Mixed plantations have been found to stabilize tree productivity under varying water conditions, but little is known about the interaction between tree biodiversity and drought on soil functioning. In this study, we focused on microbial activity by measuring soil enzyme activities (SEA). The objectives were to assess the effects of (i) drought, (ii) tree diversity and (iii) their interaction on SEA. We hypothesized that biodiversity effects would be greater along the soil profile under drought conditions. To test this, SEA related to carbon (C), nitrogen (N) and phosphorus (P) cycling were quantified in soil samples at different depths in both monocultures (*Pinus pinaster* and *Betula pendula*) and mixed plots of these two species. Half of the plots were seasonally irrigated to assess the impact of water resource on SEA. We found that N- and P-related enzymes were negatively affected by drought whereas C-related enzymes were not. Tree diversity positively influenced SEA, but this effect depended on depth. However, the results of our experiment show that negative effects of drought on SEA cannot be upset by positive effects of tree diversity. Our results highlight that changes in climatic conditions and tree diversity may have important consequences on SEA. We conclude that forest management and frequent droughts have the potential to alter microbial activity and nutrient cycling with further potential repercussions on ecosystem functioning in the Landes de Gascogne.

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