Effects of fertilization and understory removal on aboveground and belowground carbon stocks in wet and dry moorlands in southwestern France

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Abstract

Forests provide essential ecosystem services such as wood production and soil carbon storage, which can be influenced by forest management. Fertilization and understory removal (UR) are common practices set up in managed forests to reduce tree mortality and relieve trees from their main limitations, but their effects on belowground functioning and soil carbon storage are still unclear. In this study, we investigated the effects of fertilization, UR and their interaction on the carbon stored in the ecosystem and soil enzyme activities in two different moorlands in southwestern France (dry and wet moorlands) planted with maritime pines (*Pinus pinaster* Ait.). Our results showed that fertilization had a positive effect on tree biomass in the wet moorland, but did not affect soil organic carbon stocks. In contrast, UR had a positive influence on tree biomass in both sites, whereas it had a strong negative effect on soil organic carbon stocks in the dry moorland only. Furthermore, we found that carbon-related enzyme activities were not affected by fertilization, but decreased with UR in the dry moorland. When looking at the carbon pools, we found that fertilization increased total carbon stocks in the wet moorland due to an increase in aboveground biomass. In contrast, UR did not affect total carbon stocks in the dry moorland due to compensatory effects with an increase of carbon stored in the aboveground biomass coupled to a decrease of carbon stored in the soil. These results highlight the importance of adapting forest practices depending on the environmental context.

1. Introduction

Forests cover more than 30% of worldwide land area and provide multiple goods and services that are essential to mankind, such as biomass production and carbon (C) storage (Krieger 2001; Bonan 2008). Carbon in forested ecosystems is fixed *via* photosynthesis by trees and the understory vegetation, and distributed across different compartments: aboveground biomass, belowground biomass and soils. The latter is considered to be the largest C reservoir in global forests containing 44% of the organic C (estimation based on 1 m of soil depth), followed by living biomass with 42% (aboveground and belowground biomass undifferentiated), and deadwood forest floor with about 13% (Pan et al. 2011). One of the main factors limiting C fixation and inputs towards the soil is nutrient availability, notably because it strongly drives tree growth (Augusto et al. 2017). In particular, it has been shown that phosphorus (P) is one of the main elements limiting net primary productivity in many ecosystems (Hou et al. 2021), and especially in regions characterized by old or quartz-rich substrates such as the Landes de Gascogne forest in southwestern France (Achat et al. 2009; Trichet et al. 2009; Vitousek et al. 2010). Water is another major factor controlling net primary productivity in forests (Guada et al. 2016; Hartmann et al. 2018), mainly due to its direct impact on tree nutritional and hydric status (Gholz et al. 1990; Schlesinger et al. 2016). In a context of global change in which soil resources (water and nutrients) are diminishing, mainly due to an increasing water deficit (e.g., drought) and the depletion of non-renewable nutrients (i.e., phosphorus), it seems essential to implement innovative local practices to allow supply of ecosystem services such as biomass production and carbon storage in the long term.

Two of the most commonly used forest management practices are understory management and fertilization (Nohrstedt 2001; Albaugh et al. 2003; Balandier et al. 2006). The presence of understory vegetation strongly influences water availability, whereas P fertilization increases soil phosphate availability for sustaining high tree growth (Riegel et al. 1992; Trichet et al. 2009). Thus, it is often recommended to fertilize and remove the understory vegetation at plantation in order to promote tree establishment and growth (Albaugh et al. 2003; Balandier et al. 2006). However, the effect of understory removal and fertilization depends on the site conditions (Trichet et al. 2009; Chen et al. 2019). In our study region in southwestern France, trees in wet sites (hereafter referred to as *wet moorlands*) are rather limited by nutrients, whereas trees in dry sites (hereafter referred to as *dry moorlands*) are particularly limited by access to water (Jolivet et al. 2007; Trichet et al. 2008). In addition, the composition of the understory vegetation is also a key factor influencing environmental resources. For instance, common gorse (*Ulex Europaeus* L., usually abundant along with herbaceous species in wet moorlands) is a nitrogen (N)-fixing species that enhances N-inputs into the soil (Augusto et al. 2005; Vidal et al. 2019). On the other hand, ericaceous shrubs in association with ericoid mycorrhizal fungi are usually dominant in dry moorlands (Trichet et al. 1999; Fanin et al. 2022a). Their association influences the accumulation of organic matter (OM) due to the production of poor quality litter and highly melanized fungal necromass, respectively (Clemmensen et al. 2015; Fanin et al. 2022a). Furthermore, it is important to note that the removal of the understory vegetation can also modify the soil microclimate (Wang et al. 2014), as well as reduce OM inputs into the soil (Yang et al. 2018). Therefore, although fertilization and understory removal can have positive effects on nutrient and water availability for tree growth (South et al. 2006), and in turn on aboveground C biomass, the consequences on belowground C storage are relatively unknown, and may further depend on the environmental context.

Once C has entered the system, OM decomposition is one of the key factors controlling OM accumulation in forest soils. Decomposition is carried out by extracellular enzyme activities (EEAs) synthesized mainly by soil microorganisms that catalyze various reactions through the depolymerization and oxidation of simple and complex molecules within the organic matter (Burns et al. 2013; Alberti et al. 2017; Fanin et al. 2022b). The study of EEAs can thus provide essential information on mineralization processes and on the way by which soil microbial communities may control C, N and P biogeochemical cycles (Burns et al. 2013; Fanin et al. 2016; Nannipieri et al. 2018). In particular, the storage of organic C is impacted both by the nature of the C added to the environment and by the quantities of litter inputs (Jastrow et al. 2007; Augusto and Boča 2022), which may both decrease with increasing soil depth (Fierer et al. 2003). Understory removal is expected to have negative effects on the production of C-hydrolytic enzymes following a decrease in C inputs (Yang et al. 2019; Maxwell et al. 2020). Similarly, P fertilization has been shown to decrease phosphatase activities due to greater availability of phosphate in solution (Fox and Comerford 1992), and should thus increase C:P enzyme ratios due to greater energy limitation (Fanin et al. 2012; Soong et al. 2019). However, very few studies have evaluated the interactions between understory removal and fertilization on EEAs, and even less along the soil profile.

In this study, the main objective was to investigate the effects of understory removal and fertilization on C stocks in the vegetation biomass as well as in the underlying soil and soil enzyme activities. To do this, we measured tree and understory biomass and collected soil samples in two experimental pine forests located in the Landes de Gascogne, and that are representative of the study region. The first site is located in wet moorlands and its vegetation is rather diversified with a co-dominance of gorse and molinia (an herbaceous species), which typically produce a high quality litter with a low C:N ratio (Hoorens et al. 2003; Ganjegunte et al. 2005). The second site in dry moorlands is dominated by ericaceous shrubs, which typically produce nutrient-poor quality litter characterized by a high C:N ratio (Miller 1979). Within these sites, plots with P fertilization and understory removal treatments have been implemented in 2008 and the trees were measured regularly since then. Soil samples were collected during the spring in 2021 when the vegetation and soil microbial communities are most active. Following this sampling campaign, we measured EEAs related to the C cycle, organic C stocks in the soils and estimated tree growth and C stocks in the aboveground biomass. We hypothesized that (H_1) understory removal would have a greater impact on soil C stocks, EEAs and tree C biomass in dry moorlands. Indeed, water availability being the most limiting factor in these relatively dry environments, we expected that competition for water would be the main limiting factor. In contrast, we hypothesized that (H_2) P fertilization would have a greater impact on soil C stocks, EEAs and tree C biomass in wet moorlands. Indeed, nutrient availability being the most limiting factor for tree growth in these relatively wet environments (Trichet et al. 2009), we expected that P availability would be the main limiting factor. The main originality of our study is to assess simultaneously C stocks in plant biomass and in the soil in two contrasting environments in order to investigate the consequences of two common forest management practices on aboveground-belowground C pools.

2. Materials And Methods

2.1. Experimental sites and sample collection

We conducted this study in two plantation forests in southwestern France, located in the Landes de Gascogne forest. The forest region is usually described based on four site classes depending on the topography and the groundwater depth: the forest coastal dunes (~ 1 % of the forest surface area), and the inland moorlands composed of wet (~ 5 %), mesic (~30 %) and dry (15 %) moorlands (Trichet et al. 1999). The sites chosen for this study are representative of the wet moorlands and the dry moorlands, as their functioning are the most contrasted within the inland area. The main difference is due to the water table level, which is generally below 2 meters deep in dry moorlands whereas it is less than one meter deep in wet moorlands, and thus upwelling events may occur when precipitations are high. The experimental design is thus composed of two sites: the “wet moorland” site (44°50'49.18" N, 0°53'43.68" W, elevation of 48 m asl) and the “dry moorland” site (44°10'53.60" N, 1°05'45.40" W, elevation of 50 m asl). Since plantation (2008–2021 included), the mean annual temperature was 10.8°C and 11.4°C, and the mean annual cumulated rainfall was 841 mm and 1050 mm in wet site and dry site, respectively. Most of the precipitation occurs from fall to early spring, and summers are usually dry. The soil type is similar between the two study sites as it is a podzol, characterized by a coarse texture (> 90% sand) and a low soil fertility, especially when considering P pools (Trichet et al. 2009; Augusto et al. 2010). The wet moorland is dominated by perennial herbaceous species (*Molinia caerulea*) and nitrogen-fixing shrubs such as gorses (*Ulex europaeus* and *Ulex minor*), and buckthorn (*Frangula alnus*), whereas in the dry moorland the understory is largely dominated by ericaceous shrubs such as *Erica cinerea* and *Calluna vulgaris* and mosses, with small patches of other woody plants.

In each of the two experimental sites, the former pine stand was cleared, and 16 plots (surface area: wet moorland = 640 m^2 /plot; dry moorland = 900 m^2 /plot) were set up to test four treatments (control, P-fertilization, understory removal, P-fertilization combined with understory removal), repeated in four blocks. P-fertilizer was applied in the areas dedicated to the fertilization treatment (80 kg P_2O_5 ha^{-1} in the wet moorland and 60 kg P_2O_5 ha^{-1} in the dry moorland). Then, in 2008, the sites were planted with seedlings of maritime pine (*Pinus pinaster*). Seedlings were planted every 2 m along ridges that were separated from each other by 4 m-wide furrows, resulting in a tree density of 1250 trees ha^{-1}. Fertilization with P only occurred at tree plantation, whereas understory removal was performed (in the areas dedicated to the understory removal treatment) by bladed rollers during summers 2008, 2010, 2011, 2012, 2013, 2014 and 2015 in the wet moorland and during summers 2012, 2013 and 2017 in the dry moorland. Because of the tree plantation design, bladed rollers were only used in the furrows, meaning that the vegetation in the tree ridges was not controlled. Sample collection was performed in the two sites between April 22nd and 29th 2021. In each plot, we collected forest floor (layer of shed vegetative parts) using a 10 cm × 20 cm quadrant. Three samples were collected and then grouped to be representative of the plot. Then, at the same three spots, we collected a soil core down to 30 cm deep. First, the topsoil layer was sampled with a large corer (diameter = 8 cm; length = 15 cm), which was then split in two samples (0–5 and 5–15 cm). Then, the 15–30 cm layer was sampled with a thinner corer (diameter = 48 mm). The three replicates were pooled into a unique sample per layer. To account for possible bias due to temporal and spatial variability during soil collection, additional three soil cores per plot were collected in autumn 2020, winter 2020–2021, and summer 2021, and analyzed to have a robust estimation of soil C stocks at the plot scale. Soil samples were sieved at 2 mm the day after sampling and kept at -20°C. Then a first aliquot was used for extracellular enzyme activities measurements, and a second one for soil moisture and organic C assessments. In total, we collected 32 forest floor samples (2 sites × 4 blocks × 4 treatments × 1 layer), 96 soil samples collected for enzymes and soil moisture analyses (2 sites × 4 blocks × 4 treatments × 3 layers) and 384 soil samples for soil C stocks measurements in the mineral layers (4 seasons × 2 sites × 4 blocks × 4 treatments × 3 layers).

2.2 Forest floor and soil chemical analyses

Forest floor samples were sorted, dried at 50°C until weight stabilization, and then grounded and homogenized. Total C content was then determined by dry combustion on about 3–4 mg of a representative aliquot of each sample. Similarly, soil samples were dried at 50°C until constant weight and then ground to obtain finer particles of about 5 µm. Total C was determined by dry combustion on a sample of about 30–40 mg of ground soil dried at 50°C.

2.3 Ecosystem aboveground biomass

Maritime pine aboveground biomass was estimated using field measurements realized in 2020 (n = 80 trees/plot in the wet moorland site, and n = 120 trees/plot in the dry moorland site), 12 years after tree plantation. Tree height and diameter at breast height were measured in 2020. The different pine components biomass were calculated using models developed by Shaiek et al. (2011):

$$Stem\ biomass\ (kg\ tree^{-1}) = 2225 \times DBH^{2.56} \times Age^{0.19}$$

$$Branch\ biomass\ (kg\ tree^{-1}) = 1883 \times DBH^{2.47} \times Age^{-0.29}$$

$$Needle\ biomass\ (kg\ tree^{-1}) = 1916 \times DBH^{2.07} \times Age^{-0.67}$$

Additionally, during the summer of 2021, we determined the understory height and vegetation cover in each plot of the two sites by separating the species into five functional groups (ericaceous, little woody, gorse, bracken, and herbs). We then assessed the 'phytovolume' (Gonzalez et al. 2013; Vidal et al. 2021) in a 1 m large zone along two 15 m transects arrayed perpendicularly to the tree ridges and furrows. A phytosquare was established at the endpoints of each of the two transects in the tree furrow, with a total on four phytosquares in each plot. Phytovolume assessments were carried out by the same two operators in all plots, to reduce potential operator bias.

2.4 Estimation of carbon stocks in different compartments

Our objective was to assess C stocks for the following compartments: pine biomass, understory biomass and soil. Soil organic carbon stocks (SOC stocks; Mg C ha^{-1}) were calculated using equivalent soil mass for each soil layer separately using a cubic spline interpolation (Wendt & Hauser, 2013). Total soil C stocks for the top 30 cm were obtained by summing the mean SOC stocks of each layer. Forest floor C stocks were estimated by multiplying the dry weight of the sample with its total C content measured by dry combustion, and then added to soil C stocks. Pine and understory C stocks were based on the sum of aboveground and belowground C stocks. First, aboveground pine C stocks (Mg C ha^{-1}) were estimated using C content values (mg C g^{-1}) for each of the three components biomass (as described above) from Bert and Danjon (2006). Then belowground pine C stocks were assessed using allometric equations to determine taproot and coarse roots biomass from Augusto et al. (2015) and fine roots biomass from Achat et al. (2018), and then C content data from Bert and Danjon (2006) were used to convert it to C stocks. Aboveground understory C stocks (Mg C ha^{-1}) were estimated in furrows using C content data obtained using the dry combustion method (described above) on understory samples collected on both sites. Belowground understory C stocks were assessed using C content data from Ma et al. (2018).

2.5 C-related extracellular enzyme activities measurements

Extracellular enzyme activities have been assessed on samples collected during spring 2021, using soil aliquots which were stored at -20°C until measurement of the enzyme activities. We measured the potential activity of four hydrolytic soil enzymes that catalyze the degradation of organic carbon (β-1,4-

glucosidase [BG]), 1,4-β-D-cellobiohydrolase [CBH], α-1,4-glucosidase [AG], and β-xylosidase [XYL]) (Bell et al. 2013; Fanin et al. 2016). The assays were conducted by homogenizing 2.75 g of the unfrozen soil sample in 91 ml of 16.25 mM sodium acetate buffer pH 3.9 in a Waring blender for 1 min. Soil suspensions of two technical replicates were then added to 96-deepwell (800 µl) microplates using an eight-channel electronic pipette (Eppendorf Xplorer Plus, Hamburg, Germany). Additional quench control replicates of the soil suspension, 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 mixed with 200 µl of water), were included with each sample. Soil suspensions were incubated with fluorometric substrates at 25°C for 3 h. After the incubation period, plates were centrifuged at 3000 rpm for 3 min, and then 250 µl of the 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 an emission wavelength at 450 nm. Soil moisture was assessed for each sample by comparing the fresh soil weight to the dry soil weight after drying for 72 hours at 50°C. Enzyme activities were calculated as rates in nmol g^{-1} dry soil h^{-1} and then converted to quantities in mmol kg^{-1}. Finally, we summed the BGLU, CBH, AGLU, and XYL quantities as a measure for total C-related enzyme activity.

We used specific enzyme activities, defined as the enzyme activities by unit of soil organic carbon (Fanin et al. 2022b), to normalize differences in soil organic carbon contents. This allows a better comparison of soil microbial activities under different soil management practices.

2.6 Statistical analyses

All the statistical analyses were carried out using R software (version 4.2.1). A first mixed model was performed to assess the effects of fertilization (P fertilization *versus* no fertilization), understory removal (understory removal *versus* no understory removal) and their interaction on aboveground understory and pine biomass in furrows in wet and dry moorlands, separately. A second mixed model was used to assess the effects of fertilization, understory removal, soil depth (three horizons as levelled factors) and their interactions on soil organic carbon stocks and C-related extracellular enzyme activities. Blocks were included as a random factor in both models to account for the spatial structure of our experimental design, and plots were nested within the block random factor to enable the comparison of treatments within each block separately. Tukey's post-hoc tests were used to apply contrasts between treatments in each site with α = 0.05 used as the level to define significance. This was done both for total understory and pine aboveground biomass at each site. We then performed this post-hoc test to test the differences in SOC stocks between treatments for each soil layer and at each site. Finally, to test whether there were treatment effect differences on total ecosystem C pools between the two sites, we conducted the post-hoc test for each site. The descriptive data used to compare the effects of the treatments will be the means and standard error.

3. Results

3.1 Effect of fertilization and understory removal on understory aboveground biomass

Total understory aboveground biomass in furrows – that is areas in stands that can be covered by a bladed roller pulled by a tractor – was similar in plots where the understory was removed between the wet moorland (mean ± standard error = 1.80 ± 0.15 Mg ha^{-1}) and the dry moorland (1.70 ± 0.12 Mg ha^{-1}; **Fig. 1**). In contrast, in plots without understory removal, the biomass was lower in the wet moorland (3.90 ± 0.38 Mg ha^{-1}) than in the dry moorland (6.94 ± 0.73 Mg ha^{-1}). Understory vegetation of the wet moorland was more diversified than that of the dry moorland, as the five surveyed plant groups were represented in the wet moorland (i.e. gorse, ericaceous, little woody plants, bracken and herbs), whereas only two (i.e. ericaceous and little woody plants) were present in the dry moorland. The plant community in the wet moorland was dominated by herbs, representing 51% of total understory aboveground biomass (1.45 ± 0.11 Mg ha^{-1}), followed by gorse with 21% (0.59 ± 0.16 Mg ha^{-1}) and ericaceous shrubs with 18% (0.51 ± 0.13 Mg ha^{-1}). Little woody plants (0.19 ± 0.06 Mg ha^{-1}) and bracken (0.11 ± 0.07 Mg ha^{-1}) were also represented in this study site, but to a lesser extent (Fig. 1). In contrast, plant community in the dry moorland was dominated by ericaceous species, representing 99.7% of total understory aboveground biomass (4.31 ± 0.76 Mg ha^{-1}), whereas other woody plants were negligible (0.01 ± 0.003 Mg ha^{-1}) (Fig. 1).

Fertilization did not have any significant effect on understory aboveground biomass in either of the sites (Fig. 1, **Table 1**). On the other hand, understory removal had a strong and significant effect on understory aboveground biomass in the furrows, and this effect differed between sites (Fig. 1, **Table 1**). Biomass of the understory was twofold higher in the control plots (+ U) compared with the removal plots (-U) in the wet moorland (3.90 ± 0.38 Mg ha^{-1} *vs.* 1.80 ± 0.15 Mg ha^{-1}), while the biomass was 4 times higher in the control plots compared to the removal plots the dry moorland (6.94 ± 0.73 Mg ha^{-1} *vs.* 1.71 ± 0.12 Mg ha^{-1}) (Fig. 1). We did not find any interactive effect between fertilization and understory removal on understory biomass on either of the sites (**Table 1**).

Table 1 Results of the linear mixed model to test for the effects of fertilization, understory removal and their interactions on understory and pine aboveground biomass in furrows (top) and pine height in 2020 (bottom) in wet (left) and dry (right) moorlands, respectively. Significant results are in bold.

Understory aboveground biomass	Wet moorland				Dry moorland			
(Mg ha^{-1})	*num DF*	*den DF*	*F*	*p-value*	*num DF*	*den DF*	*F*	*p-value*
Fertilization (P)	**1**	**9**	2.83	0.127	1	9	0.22	0.651
Understory Removal (UR)	**1**	**9**	**41.57**	**0.0001**	**1**	**9**	**68.29**	**<.0001**
P × UR	1	9	0.43	0.530	1	**9**	0.01	0.940
Pine height								
(cm)	*num DF*	*den DF*	*F*	*p-value*	*num DF*	*den DF*	*F*	*p-value*
Fertilization (P)	**1**	**9**	**33.82**	**0.0002**	1	9	0.71	0.415
Understory Removal (UR)	**1**	**9**	**13.07**	**0.005**	**1**	**9**	**66.87**	**<.0001**
P × UR	1	9	2.30	0.164	1	**9**	0.94	0.356
Pine aboveground biomass								
(kg tree^{-1})	*num DF*	*den DF*	*F*	*p-value*	*num DF*	*den DF*	*F*	*p-value*
Fertilization (P)	**1**	**9**	**37.58**	**0.0002**	1	9	0.53	0.469
Understory Removal (UR)	**1**	**9**	**24.86**	**0.001**	**1**	**9**	**86.76**	**<.0001**
P × UR	1	9	1.39	0.262	1	**9**	0.88	0.379

3.2 Effect of fertilization and understory removal on pine growth

Twelve years after plantation, pine height was higher in the wet moorland (mean ± se = 975 ± 22 cm) compared to the dry moorland (679 ± 25 cm) (**Figure 2 a** and **b**, respectively). Fertilization and understory removal both had strong significant effects on pine height, and these effects were different between the two sites (**Figure 2 a** and **b**, **Table 1**). Fertilization significantly increased pine height of 13.2 % in the wet moorland (1035 ± 15 cm in fertilized plots *vs.* 915 ± 28 cm in unfertilized plots) (**Figure 2 a, Table 1**). On the contrary, we did not find any significant effect of fertilization on pine heights in the dry moorland (**Figure 2 b**, **Table 1**). Understory removal significantly increased pine height of 8.2 % in the wet moorland and of 29.5 % in the dry moorland (**Figure 2 a** and **b**, **Table 1**). We did not find any interactive effect of fertilization and understory removal on pine height in any of the sites (**Table 1**).

The effects of fertilization and understory removal on pine aboveground biomass were similar than on pine height. Fertilization and UR increased pine aboveground biomass by 36 % (*p-value* < 0.001) and 28 % (*p-value* = 0.001) respectively, in the wet moorland (**Figure 2 c**), whereas only UR increased pine aboveground biomass by 98 % (*p-value* < 0.001) in the dry moorland (**Figure 2 d**, **Table 1**).

3.3 Effect of fertilization and understory removal on SOC stocks

Total stocks of soil organic carbon (SOC) (i.e., forest floor + mineral soil layers) were similar between sites with an average of 44.43 ± 1.38 Mg C ha^{-1} (mean ± se) in the wet moorland and 42.29 ± 1.01 Mg C ha^{-1} in the dry moorland (**Figure 3**). The effects of fertilization and understory removal treatments differed between sites. Fertilization did not significantly affect SOC stocks in the wet nor in the dry moorland (**Table 2**). On the other hand, understory removal had no effect on SOC stocks in the wet moorland, but increased them in the wet moorland (**Figure 3**, **Table 2**). We found an interactive effect of fertilization and depth in the wet moorland (*p-value* = 0.010), that was mainly driven by higher SOC stocks in forest floor (FF) in fertilized plots (6.82 ± 0.46 Mg C ha^{-1}) than in unfertilized plots (5.47 ± 0.24 Mg C ha^{-1}) (**Figure 3**, **Table 2**). We also found that SOC stocks were interactively affected by soil depth and understory removal in the dry moorland (*p-value* <.0001); understory removal had a strong and significant negative effect on SOC stocks, and this effect was most important in the 0-5 cm soil layer (**Figure 3**, **Table 2**). Finally, we did not find any interactive effect of fertilization and understory removal in either of the sites (**Table 2**).

Table 2 Results of the linear mixed model to test for the effects of fertilization, understory removal, soil depth and their interactions on SOC stocks (top) and C-related extracellular enzyme activities (bottom) in wet (left) and dry (right) moorlands, respecively. Significant results are in bold.

SOC stocks	Wet moorland				Dry moorland			
(Mg C ha^{-1})	*num DF*	*den DF*	*F*	*p-value*	*num DF*	*den DF*	*F*	*p-value*
Fertilization (P)	1	9	0.08	0.785	1	9	0.24	0.637
Understory Removal (UR)	1	9	0.005	0.947	**1**	**9**	**9.95**	**0.012**
Depth (D)	**2**	**180**	**78.37**	**<.0001**	**2**	**24**	**165.92**	**<.0001**
P × UR	1	9	0.004	0.950	1	9	0.01	0.917
P × D	**2**	**180**	**3.90**	**0.010**	2	24	0.19	0.906
UR × D	2	180	1.32	0.270	**2**	**24**	**11.48**	**<.0001**
P × UR × D	2	180	1.26	0.289	2	24	0.03	0.994
C-related EEAs								
(mmol kg^{-1})	*num DF*	*den DF*	*F*	*p-value*	*num DF*	*den DF*	*F*	*p-value*
Fertilization (P)	1	9	3.72	0.086	1	9	2.94	0.121
Understory Removal (UR)	1	9	1.80	0.212	**1**	**9**	**17.02**	**0.003**
Depth (D)	2	24	2.94	0.072	**2**	**24**	**99.61**	**<.0001**
P × UR	1	9	0.24	0.637	1	9	0.49	0.503
P × D	2	24	1.47	0.249	**2**	**24**	**4.68**	**0.019**
UR × D	2	24	0.48	0.625	**2**	**24**	**4.84**	**0.017**
P × UR × D	2	24	0.95	0.955	2	24	0.50	0.611

3.4 Effect of fertilization and understory removal on C-related extracellular enzyme activities

C-related extracellular enzyme activities (EEAs) were 62.6 % higher in the wet moorland (1.02 ± 0.06 mmol kg^{-1}) than in the dry moorland (0.63 ± 0.05 mmol kg^{-1}) (**Figure 4**), with activities increasing with SOC content (**Appendix 1**). Fertilization alone did not have any significant effect on C-related EEAs (**Table 2**). It was only marginally significant (*p-value* = 0.086) in the wet moorland (**Table 2**), and did not have an interactive effect with any of the other tested factors in the wet moorland. However, when looking at C-related EEAs along the soil profile, it appears that fertilization had a significant negative impact limited to the 0-5 cm soil layer in the dry moorland (*p-value* = 0.019) (**Figure 4 c**, **Table 2**). Similarly, understory removal had a site-dependent effect on C-related EEAs; it showed no significant effect in the wet moorland (**Figure 4 b**, **Table 2**) whereas in the dry moorland C-related EEAs were significantly lower in plots where understory was removed (0.52 ± 0.06 mmol kg^{-1}) compared with plots in which understory vegetation was preserved (0.74 ± 0.09 mmol kg^{-1}) (**Table 2**). This effect was largely driven by differences in the distribution patterns of C-related EEAs along the soil profile (**Figure 4**). Indeed, C-related EEAs were relatively consistent along the soil profile in the wet moorland (*p-value* = 0.073) (**Figure 4**, **Table 2**), but there was a significant decreasing trend from the top to the bottom layer of the soil in the dry moorland (*p-value* <.0001) (**Figure 4**, **Table 2**).

When looking at specific enzyme activities, we found no significant effect of fertilization nor understory removal in the wet moorland (**Appendix 2**). However, understory removal had a significant negative effect on specific enzyme activities along the soil profile in the dry moorland. We also found a significant effect of depth in the dry moorland, which was characterized by lower specific enzyme activities in the surface soil layer (**Appendix 2**).

3.5 Carbon pools in wet and dry moorlands

Carbon pools (soil C stocks, understory C stocks, and pine C stocks) in the wet moorland were higher (mean ± se = 97.44 ± 3.22 Mg C ha^{-1}) than in the dry moorland (73.72 ± 1.46 Mg C ha^{-1}) (**Figure 5**). There were no significant differences between the treatments among each site (**Table 3**), although fertilization increased total C pools by 11 % in the wet moorland and slightly decreased them by 1 % in the dry moorland (**Figure 5**). Understory removal only had a small positive effect on total C pools in wet and dry moorlands (+ 4 % and + 1 % respectively) (**Figure 5**). Further analyses highlighted that differences between treatments in the wet moorland were mainly due to higher tree C stocks in fertilized plots and plots where the understory was removed (*p-value* = 0.0002 and 0.002, respectively) (**Table 3**). In the dry moorland, the differences of SOC stocks and tree C stocks between treatments cancelled each other out at the ecosystem level, as UR decreased SOC stocks but increased tree C stocks (**Figure 5**, **Table 3**). Understory C stocks were higher in plots where this layer was present (**Figure 5**, **Table 3**).

Table 3 Results of the linear mixed model applied to test for the effects of fertilization, understory removal and their interactions on the total ecosystem C pools, and on each C pool separately in wet (left) and dry (right) moorlands, respectively. Significant results are in bold.

Total ecosystem C pools	Wet moorland				Dry moorland			
(Mg C ha^{-1})	*num DF*	*den DF*	*F*	*p-value*	*num DF*	*den DF*	*F*	*p-value*
Fertilization (P)	1	9	2.41	0.155	1	9	0.05	0.820
Understory Removal (UR)	1	9	0.34	0.5727	1	9	0.07	0.792
P × UR	1	9	0.48	0.505	1	9	0.71	0.423
Soil C stocks								
(Mg C ha^{-1})	*num DF*	*den DF*	*F*	*p-value*	*num DF*	*den DF*	*F*	*p-value*
Fertilization (P)	1	9	0.01	0.933	1	9	0.11	0.746
Understory Removal (UR)	1	9	0.14	0.7186	**1**	**9**	**17.25**	**0.003**
P × UR	1	9	0.13	0.725	1	9	0.42	0.532
Understory C stocks								
(Mg C ha^{-1})	*num DF*	*den DF*	*F*	*p-value*	*num DF*	*den DF*	*F*	*p-value*
Fertilization (P)	1	9	3.81	0.083	1	9	0.22	0.651
Understory Removal (UR)	**1**	**9**	**9.89**	**0.012**	**1**	**9**	**68.24**	**<.0001**
P × UR	1	9	0.41	0.536	1	9	0.01	0.938
Tree C stocks								
(Mg C ha^{-1})	*num DF*	*den DF*	*F*	*p-value*	*num DF*	*den DF*	*F*	*p-value*
Fertilization (P)	**1**	**9**	**38.02**	**0.0002**	1	9	0.44	0.522
Understory Removal (UR)	**1**	**9**	**19.47**	**0.002**	**1**	**9**	**90.90**	**<.0001**
P × UR	1	9	2.37	0.158	1	9	1.27	0.289

4. Discussion

Through using two 13 years-old maritime pine experiments in wet and dry moorlands in southwestern France, we investigated the effects of phosphorus (P) fertilization, understory removal (UR), and their interaction on tree carbon (C) storage, soil C storage and extracellular enzyme activities (EEAs) along the soil profile. In line with our two first hypotheses, we found that the effects of P fertilization and UR were overall greater in wet and dry moorlands, respectively. These results confirmed that the effects of forest management practices on ecosystem C budget depend on environmental context and must be adapted according to the objectives of storing C in the aboveground biomass or in the soil.

4.1 (H_1) Understory removal had a greater impact in the dry moorland

The removal of the understory vegetation had a strong negative effect on understory biomass in both sites, and this even 5 and 3 years after the last bladed roller treatment in the wet moorland and the dry moorland, respectively. In agreement with our first hypothesis, we found that UR had a stronger positive effect on tree aboveground biomass in the dry moorland. This confirmed that the vegetation in the dry moorland is more water-limited than the vegetation in the wet moorland mainly because of the competition that takes place between the different plant species, as found by previous studies in the same environmental context (Demounem 1965, 1967; Lemoine 1991; Jolivet et al. 2007). The positive effect of UR on pine biomass became apparent four to five years after plantation (**Figure 2 b**). This result is in line with the recent study of Vidal et al. (2019) indicating that the competition between trees and other woody plants is at its peak two to three years after the plantation. This is also in accordance with the analysis of Balandier et al. (2006) who investigated the dynamics of the competition between trees and understory vegetation and found that large shrubs (such as ericaceous species present in the dry moorland) have a greater competitive effect on trees until they are overtopped by them.

When investigating soil C stocks, we found significantly higher soil organic carbon (SOC) stocks when the understory vegetation was present in the dry moorland, whereas understory management had no effect in the wet moorland. One possible explanation is that ericaceous shrubs can lock up nutrients in the organic matter (OM) through the production of highly recalcitrant litter (Huys et al. 2022) and fungal necromass (Clemmensen et al. 2015), and thereby reduce OM loss from the soil by saprotrophic decomposition (Fanin et al. 2022a). An increase in decomposition rates after shrub removal, associated with a decrease in C inputs, and notably in the 0-5 cm soil layer, were probably the two main mechanisms that contributed to reduced soil C stocks. Conversely, understory vegetation occurring in the wet moorland was dominated by molinia and gorse, both being more acquisitive species, with higher litter quality than ericaceous species. The relatively higher turnover and decomposition rates in the wet moorland has probably led to a lower contribution of understory vegetation to long-term soil C stocks than in the dry moorland (Huys et al. 2022), which may explain why UR had a relatively low impact in the wet moorland. These results are in agreement with the meta-analysis of Augusto and Boča (2022) showing that acquisitive species (e.g. molinia and gorse) store less SOC on average than conservative species (e.g. ericaceous shrubs), especially in infertile soils like those in our study region.

Consistent with our results on soil C stocks, we found that UR decreased C-related extracellular activities (EEAs), but only in the dry moorland. UR's negative effect on C-related EEAs was consistent along the soil gradient in this study site, and both +U and –U treatments showed a strong decrease of EEAs with soil depth, most likely due to resource scarcity or quality in deeper soil layers (Loeppmann et al. 2016). The decrease in microbial activity after UR may be due to a reduction of organic inputs from the understory layer (Grau-Andrés et al. 2020). However, the lack of effect of UR in the wet moorland suggests that ericaceous shrubs in the dry moorland also exert a direct control on microbial activity, either due to a significant effect on microbial biomass or a shift in the composition of microbial communities (Lei et al. 2021; Fanin et al. 2022a). For instance, changes in the fungi to bacteria ratio, or in the proportion of saprotrophs and mycorrhizae may generate different needs and requirements with further consequences on EEAs (Güsewell and Gessner 2009; Fanin and Moorhead 2016). These results are similar to those of Liu et al. (2022), who found a decrease of C-acquiring EEAs after UR, but contrast with those of Osburn et al. (2018) who found no effect of UR on EEAs. Taken together, these data suggest that the effect of UR on soil microbial activity are context-dependent and vary with the composition of the understory vegetation.

4.2 (H_2) P fertilization had a greater impact in the wet moorland

Considering that the Landes de Gascogne are among the P poorest soils worldwide (Achat et al. 2009; He et al. 2021), we expected that P fertilization would stimulate tree growth (Trichet et al. 2009). In agreement with our second hypothesis, P fertilization had the most important effect on tree biomass in the wet moorland. The effect of fertilization was increasingly visible since plantation, which confirms that a release in P limitation at tree plantation is a great lever to improve tree productivity in the long term in these P-limited ecosystems (Carlson et al. 2008). In line with these results, Trichet et al. (2008, 2009) found that P fertilization at plantation increased tree growth from + 23 to + 42 % at the end of the forest rotation in non water-limited sites, such as mesic and wet moorlands, while drier sites generally did not respond to P additions. These results suggest that nutrient additions can be ineffective when the water level is insufficient to support tree growth (Mendes et al. 2016) and reinforces the conclusion that the main limitation in the dry moorland is the hydric resource (Lewis and Harding 1963; Waring et al. 2019).

In contrast to our results on aboveground tree biomass, we found that P fertilization did not have a significant impact on SOC stocks in any of the sites, except for the forest floor in the wet moorland, where C stocks were higher in fertilized plots. These higher stocks were likely driven by an increase in litterfalls due to the increase in aboveground tree biomass. Indeed, using worldwide datasets, Enquist and Niklas (2002) showed that foliage biomass is positively correlated to stem diameter, therefore indicating potential higher litterfall in plots where trees are bigger (Matala et al. 2008). The absence of effect of fertilization on soil C stocks suggest only minor impacts on soil functioning. In line with this idea, P fertilization showed only a marginally significant effect on C-related EEAs in the wet moorland and a small negative effect at the horizon 0-5 cm in the dry moorland. This result is in accordance with the study of DeForest and Moorhead (2020) who found that P fertilization in a deciduous forest only decreased P-related EEAs, but had no clear effect on C-related EEAs. This result further highlights that energy limitation after UR has a greater effect on EEAs than P fertilization, especially when considering that the last P application was performed more than 10 years ago at tree plantation.

4.3 Forest management and its impact on ecosystem C pools

Our results highlight that the effects of forest management practices (i.e., understory removal and P fertilization) differ between wet and dry moorlands, notably due to the access to the water resource. In the wet moorland, P fertilization at plantation was, as expected, the most efficient treatment to increase tree aboveground biomass. It is worth noting that fertilization had little to no effect on SOC stocks and microbial activity, and thus, the C budget at the ecosystem-level was greater in fertilized plots due to an increase in C in the biomass only (i.e., when considering pine and understory) (**Figure 5**). In contrast, UR was the most efficient way to increase tree growth in the dry moorland, but it had a strong and significant negative impact on SOC stocks and microbial activity. Overall, there was a strong trade-off between C stored in the biomass and in the soil in the dry moorland, leading to a relatively stable C budget when the understory was removed (**Figure 5**).

These results indicate that a “win-win” scenario, i.e., optimizing simultaneously tree biomass and soil C storage, cannot be achieved in either of these sites, but the management practices can be adapted according to the environmental conditions to optimize tree growth while having a minimal or a neutral impact on C budget at the ecosystem scale. To attain these objectives, a further step would be to study the costs and benefits of each management practice during the whole tree rotation. For instance, P fertilization happens only once at tree plantation, whereas UR must be repeated regularly (at least until tree canopy closure), leading to a significant impact for CO_2 emissions from the use of mechanical machinery, even though the C cost of extracting and transporting P fertilizer should also be assessed. Therefore, the practice of removing the understory vegetation is not recommended in wet moorlands in the long-term (except for the few years after seedling plantation, to enable their survival) as this technique is not as effective as fertilization. On the other hand, UR seems highly useful in dry moorlands if the management objective is to improve tree survival and growth. However, if the management objective is to sequester large amounts of carbon *in situ*, the understory present in dry moorlands should not be removed, as it strongly stimulates the accumulation of SOC. Further research is needed to estimate the stability of carbon stocks in the soil over the long term, and the life cycle of carbon stored in the final wood products.

5. Conclusions

This study demonstrated that the impact of silvicultural practices on tree growth and soil C stocks depends closely on the environmental context. Although our results did not identify any “win-win” scenario in which C storage can be optimized simultaneously in the vegetation and in the soil, it appears necessary to adapt the forest management practices to local site conditions, even in a supposedly homogeneous region. Therefore, we conclude that it is necessary to assess the relative importance of different resources such as water and nutrients if we aim at optimizing tree growth and soil C stocks at the ecosystem scale.

Declarations

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Conflicts of interest/Competing interests

The authors have no competing interests to declare that are relevant to the content of this article.

Availability of data and material

The datasets generated during and/or analyzed during the current study are available from the corresponding author on request.

Code availability

RStudio version (version 4.2.1).

Authors' contributions

Conceptualization: L. Bon, L. Augusto, N. Fanin; Methodology: L. Bon, J. Gaudry, M. R. Bakker, C. Lambrot, S. Milin; Formal analysis and investigation: L. Bon, L. Augusto, M. R. Bakker, N. Fanin; Writing - original draft preparation: L. Bon; Writing - review and editing: L. Bon, L. Augusto, J. Gaudry, M. R. Bakker, C. Lambrot, S. Milin, P. Trichet, N. Fanin; Funding acquisition: L. Bon, P. Trichet, N. Fanin; Resources: L. Augusto, M. R. Bakker, N. Fanin; Supervision: L. Augusto, M. R. Bakker, N. Fanin.

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Figures

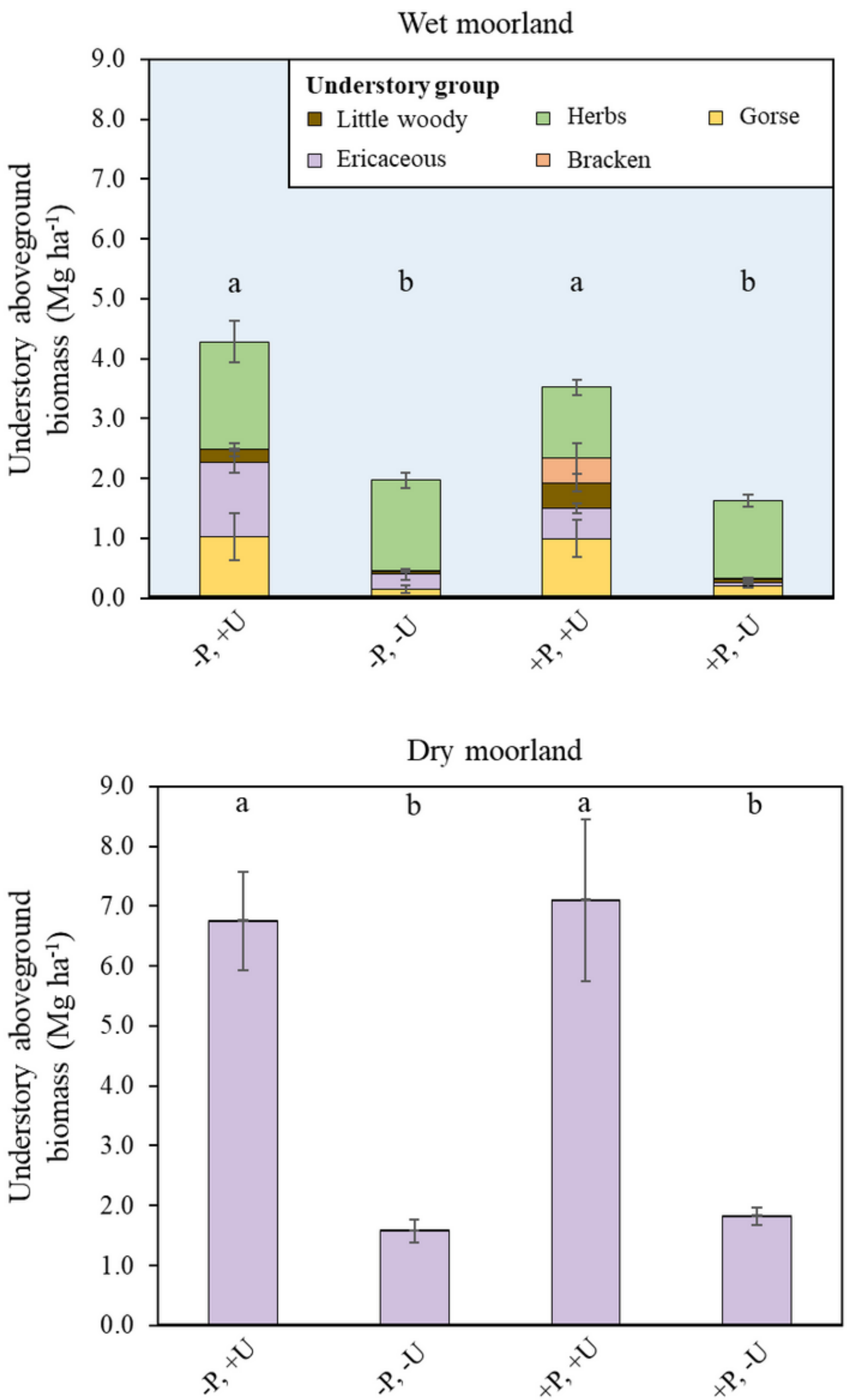

Figure 1

Aboveground biomass of the five understory groups in the furrows present on wet and dry moorlands in all the treatments (-P = unfertilized; +P = fertilized; +U = understory preserved; -U = understory removed). The lowercase letters show significant differences between treatments and were determined using a post-hoc Tukey test.

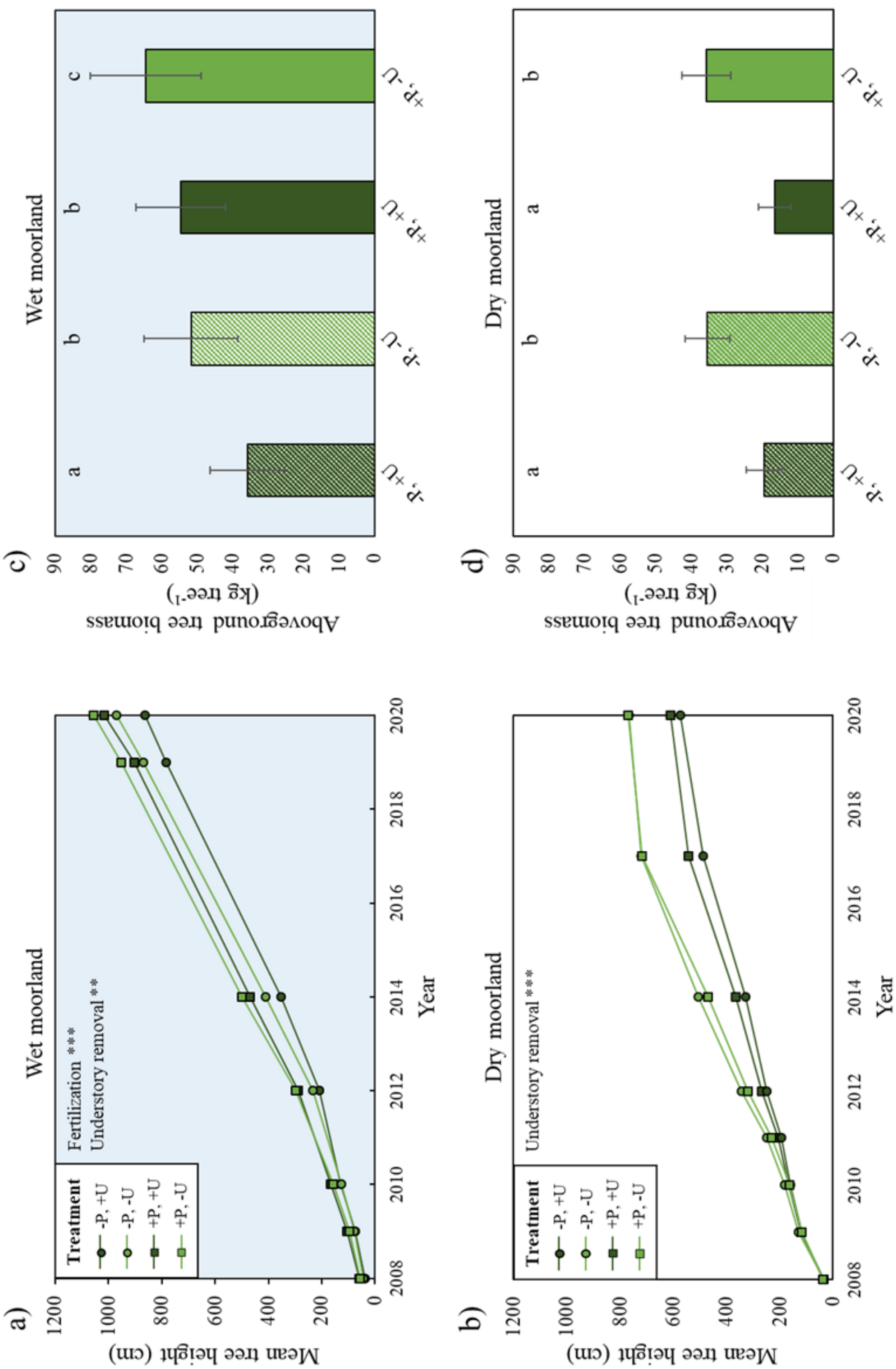

Figure 2

Interactive effects of fertilization and understory removal on pine height since plantation (a) (b), and aboveground tree biomass (c) (d). The plots represent mean tree height (cm) along the years since plantation in wet (a) and dry (b) moorlands. Significant statistical results of the linear mixed models are shown by the *, ** and *** signs. The bar plots present mean aboveground tree biomass (kg tree^{-1}) in 2020 in wet (c) and dry (d) moorlands. The lowercase letters show significant differences between treatments and were determined using a post-hoc Tukey test. Treatments: -P = unfertilized; +P = fertilized; +U = understory preserved; -U = understory removed.

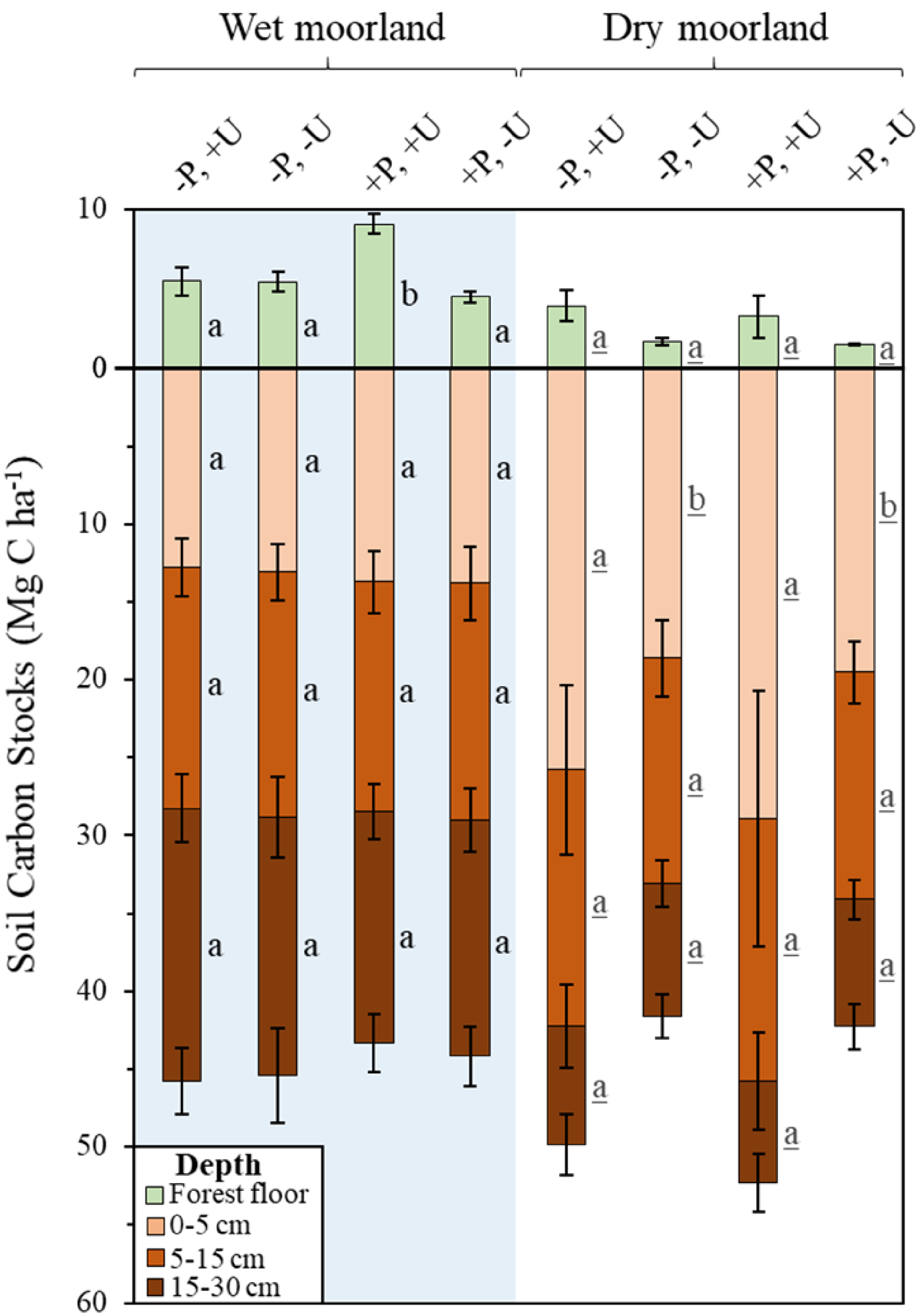

Figure 3

Interactive effects of fertilization and understory removal on soil organic carbon (SOC) stocks in wet and dry moorlands. Bar plots represent means of SOC stocks for each soil layer calculated on an equivalent soil mass basis (see Materials and Methods for more information), and error bars represent standard errors of the mean. Treatments: -P = unfertilized; +P = fertilized; +U = understory preserved; -U = understory removed.

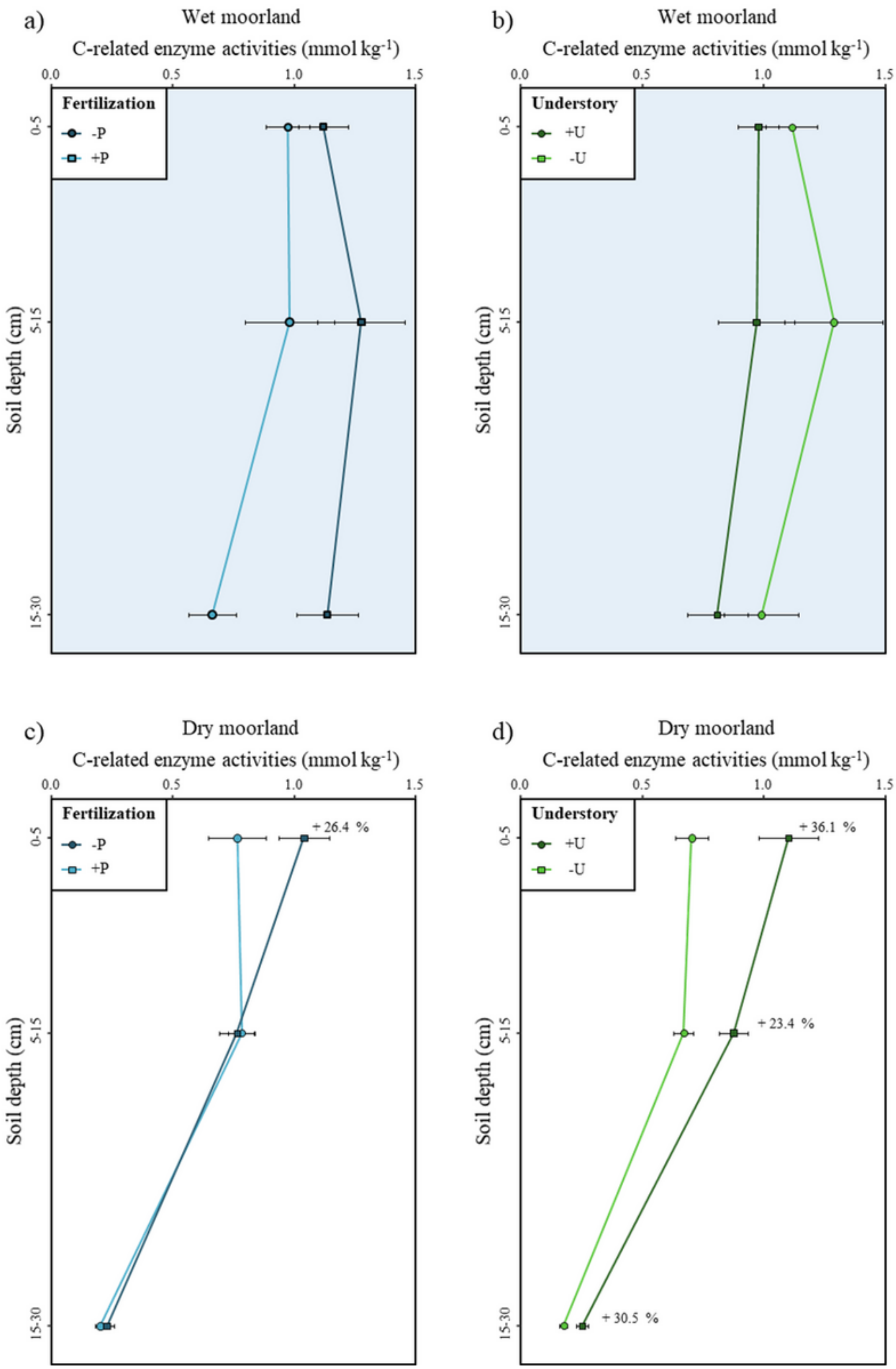

Figure 4

C-related extracellular enzyme activities (EEAs) in wet (top) and dry (bottom) moorlands depending on the fertilization (a) (c), and understory removal (b) (d) along the soil profile. Treatments: -P = unfertilized; +P = fertilized; +U = understory preserved; -U = understory removed.

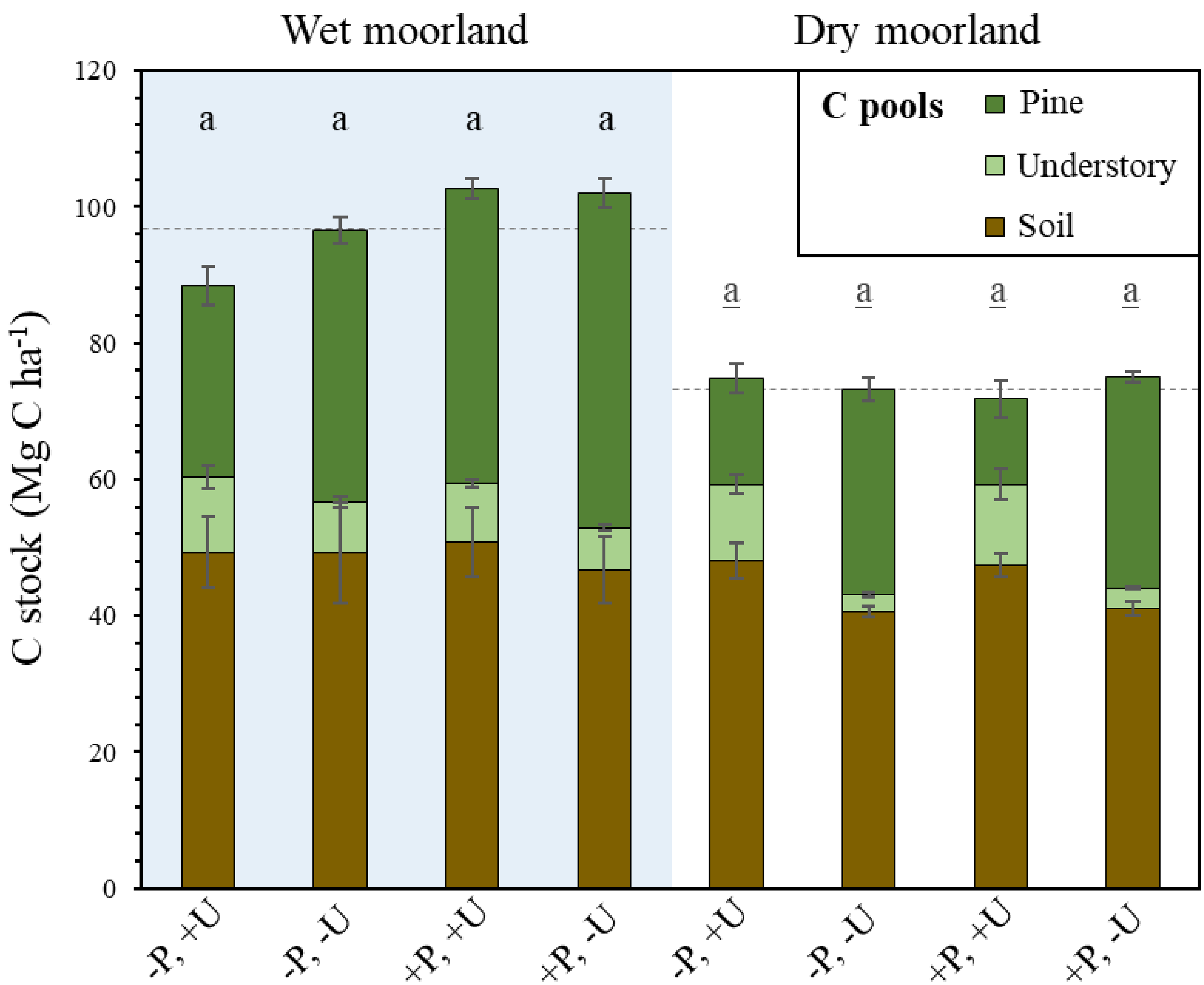

Figure 5

Interactive effects of fertilization and understory removal on carbon pools in wet and dry moorlands. Lowercase letters were determined by performing a Tukey post hoc test and show the differences between the total of the three C pools between all treatments for each site. Pine and understory C stocks consider both aboveground and belowground C stocks. The dotted line represents the mean total C stocks in wet and dry moorlands. Treatments: -P = unfertilized; +P = fertilized; +U = understory preserved; -U = understory removed.

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