RESEARCH ARTICLE

Effects of thinning and understorey removal on soil extracellular enzyme activity vary over time during forest recovery after treatment

Lixiong Zeng · Wenfa Xiao · Changfu Liu ·
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Abstract

Background and aims Soil extracellular enzyme activities (EEAs) and extracellular enzymatic stoichiometry (EES) play an important role in soil nutrient cycling processes. However, the response of EEAs and EES to forest management practices remains unclear, which hinders our understanding of the mechanisms regulating nutrient cycling.

Methods Soil microclimate, nutrients, microbial biomass, EEAs, and EES in *Pinus massoniana* plantations were investigated over 6 years after thinning (two levels: removal of 15% and 70% basal area) or understorey removal.

Results (1) Compared to the controls, the activities of β-1.4-glucosidase (BG), β-1.4-N-acetylglucosaminidase (NAG), leucine aminopeptidase (LAP) and acid phosphatase (AP) were not significantly influenced by thinning or understorey removal during the first 3 years after treatment, in the 4th year, however, understorey removal significantly reduced AP, and heavily thinning significantly increased NAG. (2) Between year 4 and year 6 after treatment, thinning significantly decreased the ratio of ln(BG) to ln(NAG+LAP) but increased the ratio of ln(NAG+LAP) to ln(AP). (3) EEAs and EES were significantly regulated by soil temperature and dissolved carbon during the first 3 years after treatment, after which microorganisms and soil total nitrogen drove the EEAs and EES.

Conclusions We found that thinning and understorey removal have similar effects on EEAs and EES. Our study suggests that nitrogen demand of soil microorganisms may change during the recovery of forests after thinning and understorey removal, which will further negatively affect EEAs and thus soil biogeochemical cycling.

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Introduction

Thinning and understorey removal are common forest management practices used worldwide to improve tree growth. However, the effects of thinning and understory removal on soil extracellular enzyme activities (EEAs) and extracellular enzymatic stoichiometry (EES) remain uncertain (Kim et al. 2016; Lull et al. 2020; Qiu et al. 2021). For instance, EEAs have been found to increase (Qiu et al. 2021), decrease (Geng et al. 2012), or remain unchanged (Kim et al. 2018) following forest thinning. EEAs can regulate nutrient cycling by affecting plant root growth and nutrient uptake (Ni et al. 2021), and by influencing decomposition and mineralization of soil organic matter (Saiya-Cork et al. 2002; Sinsabaugh et al. 2009; Sinsabaugh and Follstad Shah 2012), which will greatly affect forest growth, productivity, services, and carbon sequestration (Kim et al. 2016; Yang et al. 2018; Cheng et al. 2021; Qiu et al. 2021).

Soil extracellular enzymes produced by microorganisms and plants are ubiquitous in soil environment (Sinsabaugh et al. 2009; Allison et al. 2010). The production of soil extracellular enzymes is mainly influenced by vegetation composition (Yang et al. 2018; He et al. 2020), quality and quantity of litter input (Allison and Vitousek 2005), microclimate (Peng and Wang 2016), soil nutrient status (Sinsabaugh et al. 2009; Allison et al. 2010), and microbial communities (Yang et al. 2021). The short-term effects of thinning or understorey removal on EEAs may be caused by abrupt changes in vegetation composition, increased residue inputs, and changes in soil microclimate such as soil temperature (Cheng et al. 2021) and soil water content (Peng and Wang 2016). Higher soil temperature and higher soil moisture can improve EEAs by promoting microbial activities (Zhou et al. 2020). However, higher soil temperature may accelerate water stress by increasing evaporation and reducing substrate availability, thereby inhibiting hydrolase activity (Peng and Wang 2016). In the long term, increased growth rates of the remaining trees (Lei et al. 2021) may alter the availability of soil resources

and thus affect EEAs. The EEAs was reported to vary with the growth rate of the remaining trees and the time observed after thinning (Zhou et al. 2020). For example, acid phosphatase (AP) (Kim et al. 2016; Lull et al. 2020), β-1.4-glucosidase (BG) (Kim et al. 2016) and β-1.4-N-acetylglucosaminidase (NAG) (Geng et al. 2012) were not significantly affected by thinning during the first 4 years after thinning (Qiu et al. 2021), while all three enzymes were found to be significantly influenced by thinning in the 9th year post-treatment (Qiu et al. 2021). Therefore, continuous long-term observations are important to better understand the changes in EEAs after forest management.

As mentioned above, microorganisms may alter enzyme production in response to changes in resource availability (Allison and Vitousek 2005; Allison et al. 2010) after thinning and/or understorey removal. The change of enzyme production reflected the resource allocation theory (Allison and Vitousek 2005; Sinsabaugh and Follstad Shah 2012), that is, microbes produce more extracellular enzymes to mine the limited amounts of elements from environment (e.g., soils). Extracellular enzyme stoichiometry (EES) has thus reflected the biogeochemical equilibrium between the nutrient requirement of microbial assemblages and the nutrient availability of environment (Sinsabaugh et al. 2008, 2009) and been used to identify nutrient deficiencies and microbial nutrient requirements (Sinsabaugh and Follstad Shah 2012; Waring et al. 2014; Yang et al. 2021). A global meta-analysis by Zhou et al. (2020) showed that the resource use strategies of microorganisms differed with the stage of recovery associated with changes in tree growth rate and resource availability following forest thinning. For example, understorey removal and thinning can reduce competition for soil resources for a short period of time due to reduced plant density, but accelerated tree growth as the forest recovers (Lei et al. 2021; Cheng et al. 2021) may increase nutrient demand, which may then lead to nutrient limitation for microorganisms (Qiu et al. 2021). However, studies on long term response of EES to thinning and understorey removal are rare. The effect of thinning or understory removal on EES can be both negative and positive, especially in forest ecosystems with certain limited soil nutrient (i.e., nitrogen and phosphorus) that constrained the tree growth (LeBauer and Treseder 2008; Augusto et al. 2017; Jian et al. 2021).

On one hand, substrate input caused by treatment may provide more nutrient for microbes and relieve nutrient limitation, but accelerated tree growth with low input of litter may cause greater nutrient limitation for microbes (Qiu et al. 2021).

Pinus massoniana Lamb. is a dominant tree species with low soil phosphorus in subtropical China (Jian et al. 2021), and it covers an area of 8.04 million ha and plays an important role in C sequestration, soil and water conservation (National Forestry and Grassland Administration 2019). Thinning or understorey removal practices have been commonly used in *P. massoniana* plantations to improve forest productivity. In this study, we investigated the response of soil EEAs and EES to understorey removal and to two levels of thinning in *P. massoniana* plantations over a 6-year period. We hypothesize that (1) soil EEAs and EES respond to thinning or understorey removal similarly, because both thinning and understorey removal have similar effects on soil microclimate and nutrients, (2) the response of soil EEAs and EES to thinning or understorey removal varies with the time of stand recovery from the treatments, and (3) soil nutrients rather than soil microclimate (i.e. soil temperature and water content) altered by thinning and understorey removal determine the responses of EEAs and EES during the recovery period.

Materials and methods

Study area and thinning experiments

The study plantations are in Jiulingtou Forest Farm (30°59′N, 110°47′E), Zigui County, Hubei Province. The soil type belongs to Haplic Luvisol soil (according to Chinese soil classification) with a depth of 100–120 cm. The annual rainfall in this area is approximately 1000–1250 mm, which mainly occurred between April and September. And the annual mean temperature is 12.6 °C, ranging from −8.7 °C to 32.1 °C (Lei et al. 2021).

Three large seeded *P. massoniana* plantations established in the 1970s were chosen as the object ($n=3$ plantations). In each plantation, four plots (20×20 m each) with at least 20 m distance between any two plots were established (Lei et al. 2018). The tree density of the plantations was approximately 1700 stems per ha, and the canopy
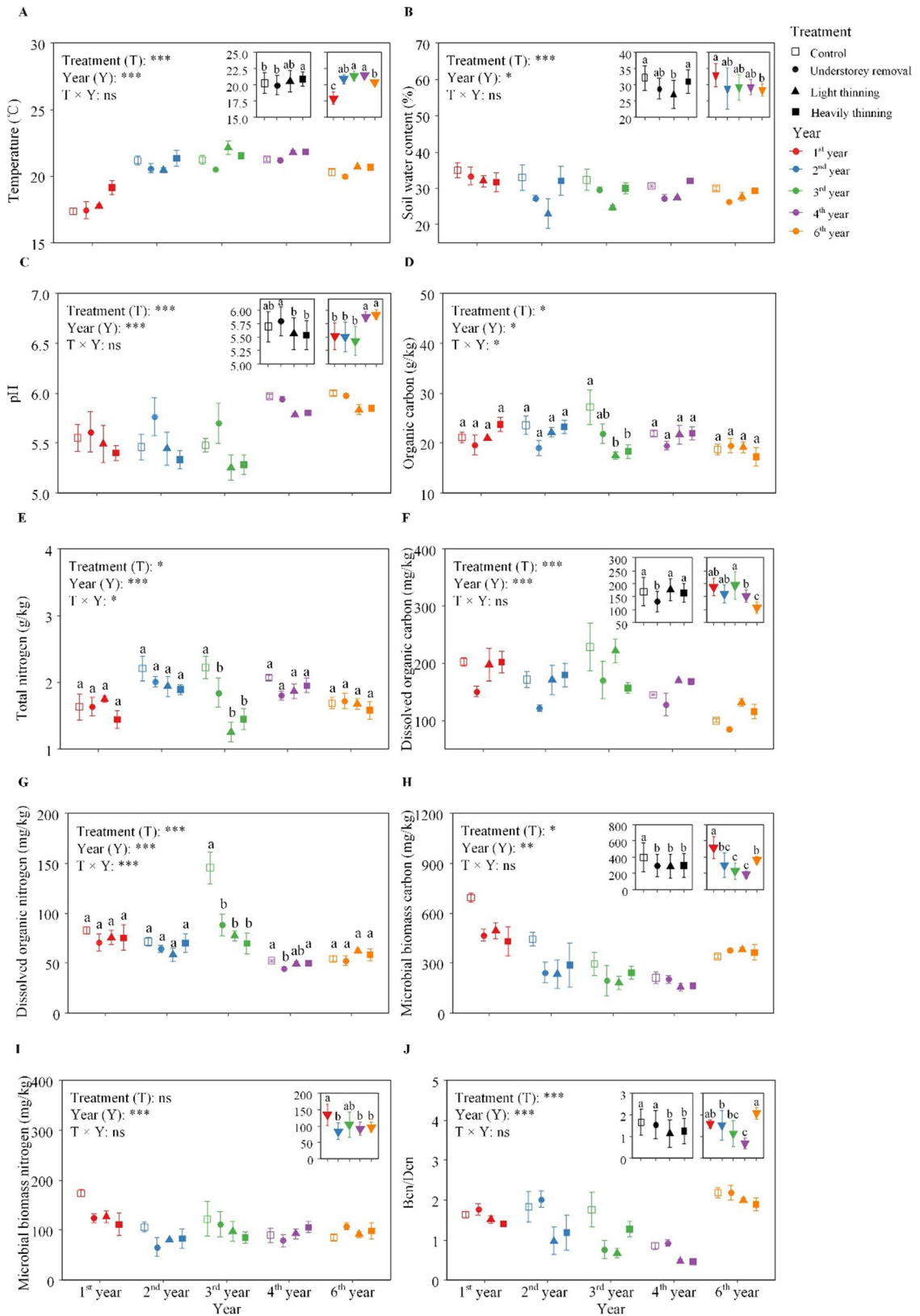
coverage was about 80% before this experiment. *P. massoniana* accounted for more than 90% of the total stem. Other coexisting tree species included *Toxicodendron vernicifluum*, *Betula luminifera* and *Cunninghamia lanceolata*. The understorey species were mainly *Pyracantha fortuneana, Litsea pungens,* and *Lespedeza bicolour*, and the coverage of the understorey vegetation is ∼49%.

In September 2013, 4 treatments were randomly conducted in four plots (one treatment for one plot) in each plantation, and all 4 treatment were repeated three times ($n=3$ plantations): (1) control (intact plots); (2) understorey removal (the aboveground parts of understorey shrubs were cut back and removed from the plots in September 2013, and repeated annually during the experimental period to keep the plots free of understorey shrubs); (3) light thinning, which reduces the stand basal area by ∼15%, and (4) heavily thinning, which reduces the stand basal area by ∼70%. Thinning treatment did not remove the understorey vegetation, and all residuals were removed from the plots (Lei et al. 2018, 2021). In the 3rd year (2016) after treatment, the annual diameter increment at breast height of the remaining trees was significantly higher in understorey removal plots (1.10 ± 0.07 cm), light thinned (1.12 ± 0.06 cm) and heavily thinned plots (1.04 ± 0.12 cm) than in control plots (0.96 ± 0.09 cm) (Lei et al. 2021).

Soil sampling

In summer (June or July) of 2014, 2015, 2016, 2017 and 2019 (5 sampling years), i.e., in the 1st (2014), 2nd (2015), 3rd (2016), 4th (2017) and 6th (2019) years after treatment, nine randomized soil samples (0–10 cm depth) were collected using an auge in each plot after removing the upper organic layer. And then all nine soil samples taken in each plot were fully mixed and stored in a refrigerator at 4 ℃. The sampling locations were marked to avoid repeated sampling at the same location, and 60 samples were analysed.

The soil temperature at a soil depth of 10 cm in each plot was measured hourly using a EM50 Data logger with five sensors (Decagon Devices, WA, USA). The mean soil temperature in each sampling month was calculated for further analysis.

Measurement of soil properties

Each mixed soil sample was divided into two parts for the measurement of soil properties. One part was air-dried and passed through a 2 mm mesh sieve for pH analysis and through a 0.149 mm mesh sieve for soil organic carbon (SOC) and total nitrogen (TN) analysis. The remaining part was passed through a 2 mm mesh sieve and divided into two subsamples: one subsample was stored at 4 ℃ for measurement of soil water contents and soil extracellular enzyme activities (EEAs), and the other subsample was stored at –20 ℃ for soil microbial biomass carbon/nitrogen (MBC/MBN) and dissolved organic carbon/nitrogen analysis. All analyses were completed within 1 month.

The soil water content was measured using oven-dried method within 1 day after sampling. Soil pH was analysed in a 1:2.5 soil-water suspension (Huang et al. 2014). Dichromate oxidation method was used to quantify soil organic carbon (Nelson and Sommers 1996), and the Kjeldahl method was used to analyse soil total nitrogen, which is the sum of the organic bounded nitrogen group and the ammonium-nitrogen (Bremmer 1996). Dissolved organic carbon and dissolved organic nitrogen were determined by K_2SO_4 extraction (Jones and Willett 2006): soil-to-solution ratio of 1:5 (w/v) was extracted with 0.5 M K_2SO_4 shaking during 1 h at 20 ℃, and the dissolved organic carbon and dissolved organic nitrogen in the extracts were detected with an elemental analyzer (elementar vario Macro cube, Germany). Chloroform fumigation extraction was used to determine MBC and MBN (Brookes et al. 1985; Vance et al. 1987). The Bcn/Dcn ratio (Bcn is the ratio between MBC and MBN, Dcn is the ratio between dissolved organic carbon and dissolved organic nitrogen) was also calculated (Cui et al. 2018).

Enzyme assay

B-1.4-glucosidase (BG, a C-acquiring enzyme), β-1.4-N-acetylglucosaminidase (NAG, an N-acquiring enzyme), leucine aminopeptidase (LAP, also an N-acquiring enzyme) and acid phosphatase (AP, an organic P-acquiring enzyme) were measured using fluorometric protocol within a week after sampling (Saiya-Cork et al. 2002; German et al. 2011). And 4-methylumbelliferyl substrates were used for measurement of BG, NAG and AP, and L-leucine-7-amido-4-methyl substrates were used for measurement of LAP. First, soil slurries were prepared by homogenizing fresh soils (equal to 1 g dry soil) in 125 ml 50 mM acetate buffer (pH = 5.5) for one minute. Then, 96-well microplates were used (8 replicate wells per sample per assay). The analysis of each sample included eight replicate wells for each enzyme (200 µl soil slurries + 50 µl substrate), a blank (200 µl soil slurries + 50 µl buffer), a negative control (200 µl buffer + 50 µl substrates), and a quench standard (200 µl soil slurries + 50 µl standard substrate). After incubated in the dark at 25 °C for 3 h, a 10 µl aliquot of 1 mol·L^{-1} NaOH was added to each well of microplates to stop the reaction. At last, fluorescence was measured using a fluorometer (SpectraMax i3x, Molecular Devices, Beckman Kurt, America) with 365-nm excitation and 450-nm emission filters (Jian et al. 2021). EEAs were normalized in units of nmol·h^{-1}·g^{-1} dry soil (German et al. 2011). Soil extracellular enzyme stoichiometry (EES) was determined using ln(BG):ln(NAG+LAP), ln(NAG+LAP):ln(AP) and ln(BG):ln(AP), respectively (Sinsabaugh and Follstad Shah 2012).

Statistical analysis

The effects of treatment, recovery time (sampling year) after treatment and their interactions on EEAs, EES and other variables were evaluated by Linear mixed models. After normality testing and Mauchly's test, treatment and year were used as fixed factors in each year, and interannual variations (year-to-year) of EEAs, EES and other variables in relation to treatment were analysed. In all these analyses, plantation (i.e., site) was considered as a random factor (Fig. 1). Differences among the levels of factors were determined by post hoc multiple comparisons. Linear mixed models

and post hoc multiple comparisons were performed using "nlme" and "multcomp" R packages.

Redundancy analysis (RDA) was used to analyse the relationships between EEAs or EES and other variables. The "ordiR2step" function of the "vegan" R package was used to conduct a forward-selection procedure on soil properties to determine which soil property significantly dominated the variations in EEAs and EES ($P < 0.05$). Statistical analyses and graphics were processed using R software (version 4.1.2, R Core Team 2021), and $\alpha < 0.05$ level was used to determine the statistical significance.

Result

Changes in soil properties

Treatment (i.e., thinning or understorey removal) significantly affected soil temperature, soil water content, pH, dissolved organic carbon, microbial biomass carbon (MBC), and the Bcn/Dcn ratio (Fig. 1): Compared to the control, heavily thinning significantly increased soil temperature, light thinning significantly decreased soil water content, and understorey removal significantly decreased dissolved organic carbon. Both thinning and understorey removal significantly decreased MBC, and only thinning but not understorey removal significantly decreased the Bcn/Dcn ratio. Soil temperature, soil water content, pH, dissolved organic carbon, MBC, microbial biomass nitrogen (MBN) and Bcn/Dcn were all significantly affected by sampling time (year) (Fig. 1).

Treatment x time interactions significantly affected soil organic carbon (SOC), total nitrogen (TN), and dissolved organic nitrogen (Fig. 1). Compared to the control, thinning significantly decreased SOC, TN, and dissolved organic nitrogen (Fig. 1D, E and G) in the 3rd year, and understorey removal significantly decreased dissolved organic nitrogen in the 4th year.

Changes in soil EEAs and EES

Time alone significantly affected BG and LAP (Fig. 2A and C). The effects of treatments (i.e. thinning, understorey removal) on AP and NAG varied across years, showing a significant treatment x time interaction (Fig. 2B and D). Compared to the control, a significant effect of treatments was found

in the 4th year: understorey removal significantly decreased AP (Fig. 2B), while heavily thinning significantly increased NAG (Fig. 2D).

Both treatments and time showed significant effects on both the ln(BG):ln(NAG+LAP) ratio and the ln(NAG+LAP):ln(AP) ratio (Fig. 3A and C). Compared to the control, heavily thinning increased the ln(NAG+LAP):ln(AP) ratio but decreased the ln(BG):ln(NAG+LAP) ratio. A higher ln(NAG+LAP):ln(AP) ratio and a lower ln(BG):ln(NAG+LAP) ratio were found in the 2nd and the 3rd year. Both thinning and understorey removal significantly decreased the ln(BG):ln(NAG+LAP) ratio but increased the ln(NAG+LAP):ln(AP) ratio in the 4th and 6th year (Fig. S1). The ln(BG):ln(AP) ratio was only significantly influenced by recovery time (Fig. 3B).

Relationships between the EEAs and EES of soil and soil properties

Redundancy analysis showed that soil properties can only explain 10.93% of the variation in EEAs and EES. When we divided the 6 observation years into two recovery stages based on the changes in EEAs, EES and Zhou et al. (2020), i.e., an early phase of 1–3 years and a middle phase of 4–6 years after treatment, we found that soil properties explained 53.92% of the variation in EEAs and EES in the early phase (Fig. 4A). Dissolved organic carbon was positively correlated with both EEAs and EES (except for ln(NAG+LAP):ln(AP) ratio) and it could explain 21% of the variation in EEAs and EES. Soil temperature was another important factor, explaining 12% of the variation. Soil temperature was positively correlated with ln(NAG+LAP):ln(AP) ratio, but negatively correlated with EEAs (except LAP) and other EES (Fig. 4A). Soil properties explained 72.56% of the variation in EEAs and EES in the middle recovery phase (Fig. 4B). Both MBC and MBN were positively correlated with BG, ln(BG):ln(NAG+LAP) ratio, and ln(BG):ln(AP) ratio, while MBC was negatively correlated with NAG, LAP and ln(NAG+LAP):ln(AP) ratio (Fig. 4B). MBN was also positively correlated with AP and NAG. TN showed a positive correlation with nitrogen-related enzymes and ln(NAG+LAP):ln(AP) ratio (Fig. 4B).
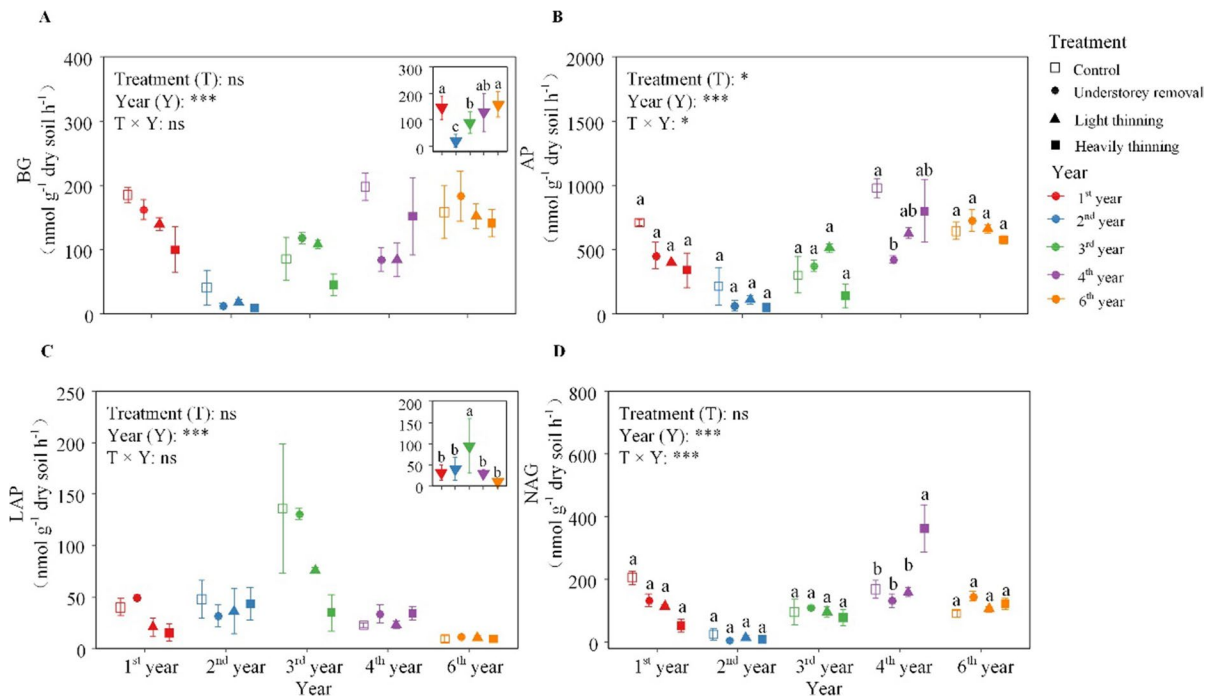
Fig. 2 Mean values (± SE; $n=3$) of EEAs in control, understorey removal, light-thinned and heavily thinned plots over time after treatment. Asterisks represent p values for the main effects of treatment, year, and their interactions on soil environmental variables (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$). In the case of a significant interaction, letters above points were added to indicate significant differences among levels of treatment in each year ($p \leq 0.05$). In the case of significant treatment or time effects only, the results of the post hoc test were inserted as small figures

Discussion

### Effects of thinning or understorey removal on EEAs and EES over time

In line with our 1st hypothesis, we found significant lower soil microbial biomass (MBC) in the thinned or understorey-removed plots than in the controls. These may be the result of decreased litter input due to reduced plant density by thinning or understorey removal (Campbell et al. 2009) on the one hand, and increased soil temperature and decreased soil water content caused by treatment (Fig. 4) on the other hand (Peng and Wang 2016).

Changes in EES are often used to determine the nutrient requirements of microorganisms (Sinsabaugh et al. 2008; Waring et al. 2014; Yang et al. 2021). Soil organic matter and nutrient availabilities are closely correlated with EEAs (Sinsabaugh 2010), which has also been confirmed by significant effects of dissolved organic carbon and TN on soil enzyme

activities in our study (Fig. 4). When the supply of nutrients is consistent with the demand of microbes, microbes tend to reduce the costs of enzyme production and inhibit enzyme activity; otherwise, enzyme production is accelerated (Allison and Vitousek 2005; Burns et al. 2013).

Again, consistent with our 1st hypothesis, we found that both thinning and understorey removal did not have significant effect on EEAs and EES during the first three years following treatment (Table S1). There might be sufficient nutrients for microbes, as similar dissolved organic carbon per unit of microbial biomass (expressed as a ratio between dissolved organic carbon and MBC) was found in all treatments. However, reduced litter input (Lei et al. 2021) and thus N content from litter (Table S2) in the 3rd year caused by heavily thinning, might lead to significantly increased NAG in the 4th year, which helps to degrade relatively complex compounds (i.e., roots residues) to obtain N (Burns et al. 2013; Sinsabaugh et al. 2009). Meanwhile, we also found that both thinning

Fig. 3 Mean values (± SE; $n = 3$) of EES in control, understorey removal, light-thinned and heavily thinned plots over time after treatment. Asterisks represent *p* values for the main effects of treatment, year and their interactions on soil environmental variables (* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$). In the case of a significant interaction, letters above points were added to indicate significant differences among levels of treatment in each year ($p \leq 0.05$). In the case of significant treatment or time effects only, the results of the post hoc test were inserted as small figures
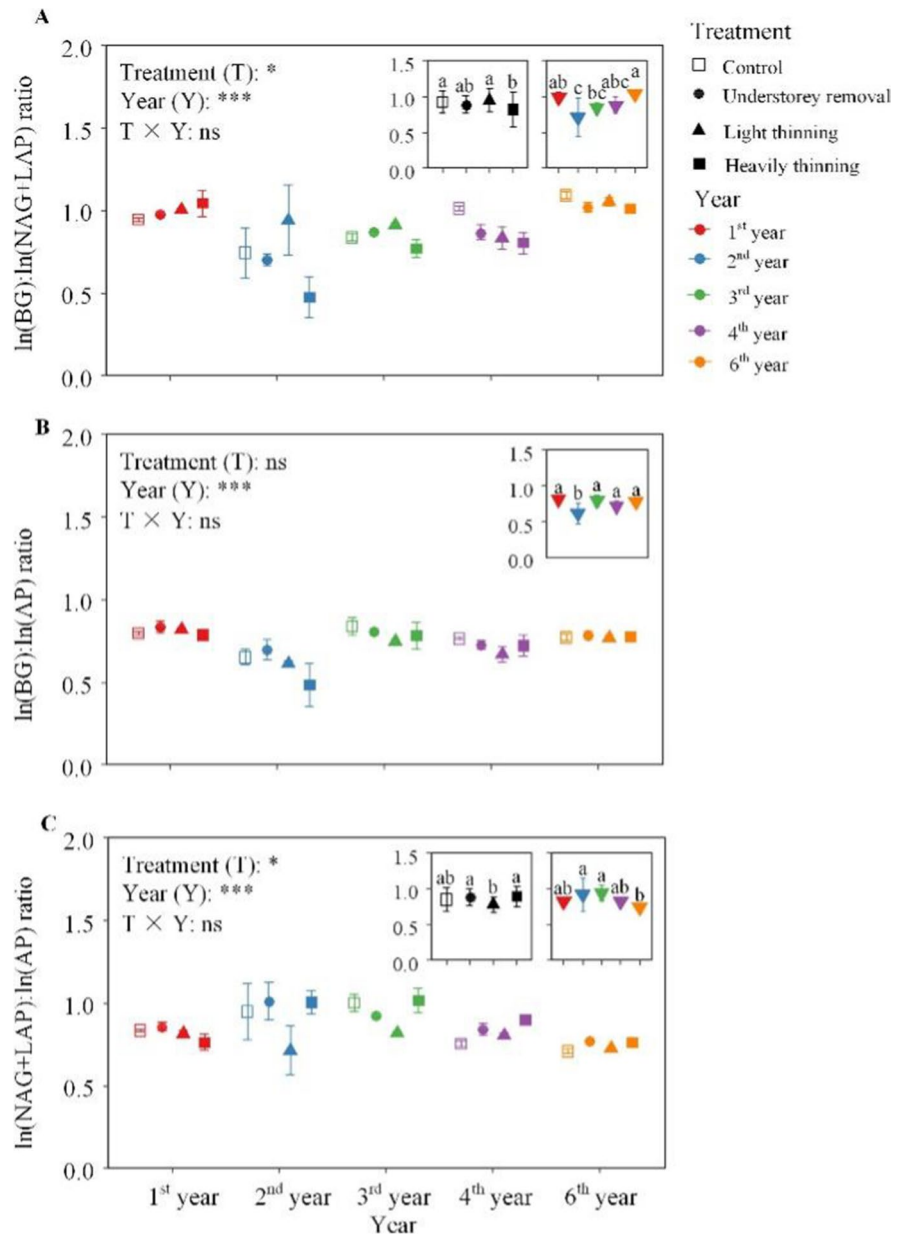
and understorey removal decreased AP in the 4th year (Fig. 2B), which may be due to a decrease in AP produced by the removed plants (Allison et al. 2010), as well as a decrease in N input in the 3rd year, since AP production requires sufficient N availability (Treseder and Vitousek 2001).

Similarly, we also found that both thinning and understorey removal decreased the ratio of ln(BG) to ln(NAG + LAP), and increased the ratio of ln(NAG + LAP) to ln(AP) in the 4th and 6th year after treatment (Fig. S1), which may be related to a decrease of needle litter TN, soil TN and soil dissolved organic nitrogen occurred in the 3rd year (Table S2, Fig. 1). According to resource allocation theory and the biogeochemical equilibrium model ($Scn = \frac{Bcn}{Dcn} \times \frac{1}{ln(BG):ln(NAG+LAP)}$) (Sinsabaugh and Follstad Shah 2012; Cui et al. 2018), microbes regulate their enzyme production to maintain a
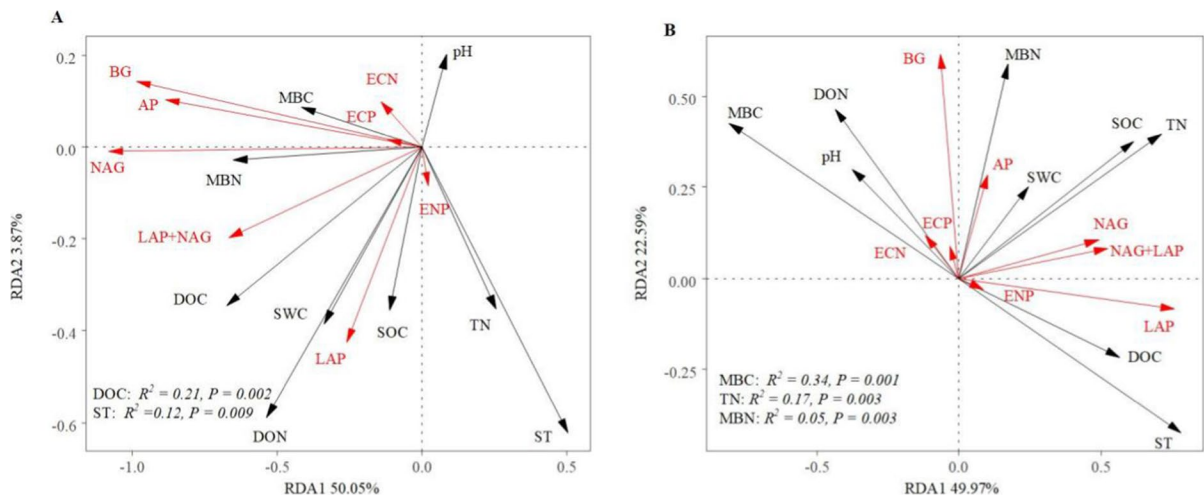
Fig. 4 Redundancy analysis (RDA) to determine the effects of soil properties (black arrows) on EEAs and EES (red arrows) at 1–3 years post treatment (**A**) and 4–6 years post treatment (**B**). Significant influencing factors are also shown. ST: soil temperature; SWC: soil water content; pH: pH value of soil; SOC: soil organic carbon; TN: soil total nitrogen; CN: soil carbon to nitrogen ratio; DOC: soil dissolved organic carbon; DON: soil dissolved organic nitrogen; MBC: soil microbial biomass carbon; MBN: soil microbial biomass nitrogen. ECN: ln(BG):ln(NAG+LAP); ENP: ln(NAG+LAP):LN(AP); ECP: ln(BG):ln(AP)

relatively stable N supply. A significantly lower Bcn/Dcn (Fig. 1J) ratio accompanied with significant positive relationship between ln(BG):ln(NAG+LAP) ratio and Bcn/Dcn ratio (Fig. S2) indicated that microbes regulated ln(BG):ln(NAG+LAP) ratio in response to decreased soil dissolved organic nitrogen occurred in the 3rd year (Cui et al. 2018).When nitrogen is lower, microbes produce phenol oxidase to mineralize poorly available C sources to obtain N (Schimel 2003; Manzoni et al. 2012). Consistently, we found higher phenol oxidase in thinning or understorey removal plots in this period (Fig. S3). Our results also suggest that microbes regulate nutrient utilization strategies by altering the production of EEAs to adapt to treatment-induced nutrient changes (i.e., N in our study).

We found strong effects of recovery time on EEAs and EES, which supports our hypothesis 2. Similarly, Zhou et al. (2020) found that the response of EEAs to thinning changed with recovery stages based on a global meta-analysis. The findings of Zhou et al. (2020) emphasized that the input of thinning residues, high soil temperature and soil water content stimulated the productions of EEAs during the first

3 years after treatment. In our study, the above ground residues were removed from the plots, and only root residues were left in the treated plots. More persistent root residues (Wu et al. 2018) combined with reduced litter inputs (Lei et al. 2021) reduced the substrate for both microbial growth and the production of EEAs. Therefore, EEAs tended to decrease with significantly decreased soil microbes during the first 3 years (Figs. 1H and 2). The reduced substrate might still meet the demand of surviving microbes, so there was no significant changes in EES (Kim et al. 2016).

Due to decreased labile substrate inputs (i.e., above ground litter) in the previous years, microbes adjusted their enzyme production to use recalcitrant substrate, as shown by increases in both phenol oxidase (Fig. S3) and NAG (Fig. 2D) in the heavily thinning plots with the minimum litter input in the 4th and 6th year (Schimel 2003; Manzoni et al. 2012; Burns et al. 2013). In addition to the effects of litter quantity and quality, the growth rate of the remaining trees might also regulate EEAs and EES through the utilization of soil nutrients. Earlier studies showed that thinning increased the uptake of all elements (Mitchell et al. 1996) and nitrogen uptake was highly correlated with tree growth

(Carlyle 1998) after thinning, we also found significant negative correlation between increment of diameter at breast height and soil N (Fig. S4). Accelerated tree growth (Lei et al. 2021), decreased litter fall and litter N inputs (Fig. S4, Table S2) in thinning or understorey removal plots in the previous years led to decreased soil N and then caused both lower AP and ln(BG):ln(NAG + LAP) ratio but higher ln(NAG + LAP):ln(AP) ratio in the following years. Our results suggest that changes in key nutrients that regulate soil microbes may cause significant shifts in EEAs and EES. Considering the effect of EEAs and EES on mineralization process, it is speculated that N may be the key factor limit growth of trees in the following years.

Effects of soil environment on EEAs and EES

Soil variables explained only ~11% of the variations in EEAs and EES over the entire study period. However, soil variables explained more than 50% of the variation in EEAs and EES at different recovery stages after treatment, i.e., an early phase of 1–3 years and a middle phase of 4–6 years after treatment. Consistent with our hypothesis 3, soil microbes had the greatest effects on EEAs and EES, followed by soil nutrients, and soil microclimate (soil temperature) (Fig. 4). Significant effects of soil microbes and soil nutrients on EEAs and EES in forests have also been reported by Qiu et al. (2021).

Enzyme production requires large amounts of nutrients (Sinsabaugh et al. 2009), thus we found that soil nutrients rather than soil microclimate affected enzyme production, and that dissolved organic carbon was positively correlated with EEAs and MBC in early recovery phase after treatment (Fig. 4A). In the middle recovery phase, significant decreases in soil N availability in the 3rd year after treatment suggested a high N demand for microbes in thinning or understorey removal plots, thus soil N became an important nutrient regulating EEAs and EES (Fig. 4B). This high N demand for microbes is further enhanced by the N-investment into enzyme production (Schimel 2003), therefore we found that the relationship between soil microbes (MBC) and N-acquiring enzymes shifted from a positive correlation in the early recovery stage to a negative correlation in the middle recovery stage (Fig. 4). This result suggests that there is a trade-off between soil enzyme production and microbes to optimize the acquisition

of soil N in response to availability of soil nutrients. Our findings are consistent with the resource allocation theory that the relationship between EEAs and available nutrients may change when the response of microorganisms to changes in available nutrients reaches a boundary (Sinsabaugh et al. 2002; Wallenius et al. 2011; Xu et al. 2017).

The significant effects of soil temperature on EEAs and EES were found only in the early recovery phase (Fig. 4A). Higher soil temperature may reduce substrate availability and decrease soil water availability due to increased evaporation, thereby inhibiting EEAs (Peng and Wang 2016), as indicated by the negative correlation between soil temperature and EEAs (except LAP) (Fig. 4A). Soil temperature may indirectly regulate LAP through its effect on soil water content, as LAP has been observed to be positively correlated with soil water content in our study and elsewhere (Liao et al. 2016).

Conclusions

In this study, we found that thinning or understorey removal had similar time-dependent effects on soil extracellular enzyme activity and soil extracellular enzymatic stoichiometry during post-treatment recovery of forests. This is mainly because, regardless of the magnitude of biomass reduction, thinning or understorey removal similarly reduce the aboveground biomass of the stand and undergo a similar process of stand recovery after treatment. Decreased ratio of ln(BG) to ln(NAG + LAP) and increased ratio of ln(NAG + LAP) to ln(AP) suggest that nitrogen demand of soil microorganisms may change during the recovery of forests after thinning or understorey removal, which will negatively affect EEAs and thus soil biogeochemical cycling, and ultimately influence forest growth rate and productivity. These implications suggest that the above ground residues should be left in stand, which would be beneficial to nutrient supply for both trees and microbes, and thus lighten the effect of thinning or understorey removal on soil nutrient and tree growth.

and critical comments on this manuscript. We also thank the National Forest Ecosystem Station of Three Gorges Reservoir in Zigui County for their support in our field work.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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