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ORIGINAL PAPER

Thinning affects microbial biomass without changing enzyme activity in the soil of *Pinus densiflora* Sieb. et Zucc. forests after 7 years

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Abstract

• ***Key message*** **Thinning increased microbial biomass but did not alter enzyme activities in the soil of *Pinus densiflora* Sieb. et Zucc. forests in South Korea. This effect of thinning was larger under a relatively heavy thinning intensity, but there was divergence in the magnitude between sites.**

• ***Context*** The balance between microbial biomass accumulation and enzymatic C and N assimilation determines the level of bio-available C and N. However, the effects of thinning on these parameters remain contradictory and unconfirmed.

• ***Aims*** The effects of thinning intensity on microbial biomass and enzyme activity were assessed in the soil of *Pinus densiflora* Sieb. et Zucc. forests in South Korea.

• ***Methods*** Un-thinned control and 15 and 30% basal area thinning treatments were applied to two 51- to 60-year-old *P. densiflora* forests with different management histories, topographies, rainfall amounts, and soils. Seven years after thinning, microbial biomass and activities of *N*-acetyl-glucosaminidase, β-glucosidase, cellobiohydrolase, β-xylosidase, phenol oxidase, and peroxidase were measured before and after seasonally concentrated rains and at 0–10 cm depth.

• ***Results*** Microbial biomass was generally highest under the 30% basal area thinning and lowest under the control, and was positively correlated to total soil C and N. The increase in microbial biomass was lower at the site displaying sandier, drier, and more acidic soils and retaining smaller amounts of thinning residue. Conversely, thinning had no significant effect on activities of all enzymes at both sites in both periods.

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Contribution of the co-authors
Seongjun KIM led the present study, including sampling, experiments, data analyses, and manuscript writing. Guanlin LI, Seung Hyun HAN, and Hyun-Jun KIM participated in data analyses and manuscript writing. Choonsig KIM, Sang-Tae LEE, and Yowhan SON coordinated the research project and supervised overall processes for the present study.

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• ***Conclusion*** Thinning can promote accumulation of microbial biomass without significant change in enzyme activities participating in the assimilation of C and N. This effect of thinning tended to increase with thinning intensity but differed in magnitude between sites.

Keywords Enzyme assay · Forest management · Korean red pine · Soil microbes

1 Introduction

Thinning to remove deformed trees and reduce competition among trees can significantly alter forest ecosystem functioning (Baena et al. 2013; Dannenmann et al. 2006; Smolander et al. 2015). For example, thinning alters both aboveground and belowground organic matter dynamics by stimulating growth of remaining vegetation and altering litter and root production of remaining trees and understory vegetation and by adding thinning residues into the soil surface (Kim et al. 2016b; Smolander et al. 2015). Moreover, the reduced canopy cover and transpiration after thinning alter microclimate including light, temperature, and soil water availability (Chen et al. 2015; Son et al. 2004).

Soil microbes control numerous processes, such as heterotrophic respiration and nutrient mineralization or immobilization, and act both as sources and sinks of C and nutrients (Hodge et al. 2000; Schimel and Bennett 2004). Although microbial biomass contains only a small fraction of total soil C and nutrient pools, there is a rapid turnover of elements contained in microbial biomass (Bonde et al. 1988). Microbes also secrete soil enzymes promoting the assimilation of C and N by trees from soil organic matter (e.g., *N*-acetyl-glucosaminidase, β-glucosidase, cellobiohydrolase, β-xylosidase, phenol oxidase, and peroxidase). The balance between microbial biomass accumulation and enzymatic depolymerization governs the level of plant-available soil nutrients (Schimel and Bennett 2004); accordingly, it determines the composition and productivity of the plant community (Van Der Heijden et al. 2008).

Changes in organic matter balance and microclimate due to thinning can stimulate soil microbial biomass and enzyme activity. Unharvested thinning residues and dead roots and mycorrhizae can add organic C and N into soils and increase soil C/N ratio, and thereby control substrate availability for microbial biomass accumulation and enzyme activation (Adamczyk et al. 2015; Boerner et al. 2008; Wang et al. 2016). Increased soil temperature and moisture in thinned forests can alter C and N utilization pattern by soil microbes (Bolat 2014; Chen et al. 2015). However, the general effects of thinning on microbial biomass and enzyme activity remain uncertain: previous studies detected either increase or decrease (Bolat 2014; Chen et al. 2016; Dannenmann et al. 2006; Geng et al. 2012; Thibodeau et al. 2000; Wang et al. 2016) as well as no change in soil microbial biomass and enzyme activity after thinning regardless of post-thinning alterations in soil temperature and moisture (Boyle et al. 2005; Maassen et al. 2006; Kim et al. 2016c; Tan et al. 2008).

Thinning intensity may be one of the reasons for the contradictory results regarding soil microbial biomass and enzyme activity in thinned forests. A severe thinning intensity can leave larger amount of slashes as well as dead root and mycorrhizae than a comparatively light thinning intensity (Ko et al. 2014; Smolander et al. 2015). It can also provide more organic matter originating from understory vegetation by increasing light and moisture availability on the forest floor than a lighter thinning intensity (Chen et al. 2016). These differences due to thinning intensity can result in large variations in lignocellulosic and chitin substrates being added to soil C and N pools, which can have differential effects on microbial biomass and enzyme activity (Adamczyk et al. 2015; Chen et al. 2015; Kim et al. 2016c).

The objective of the present study was to test the effects of thinning on microbial biomass and enzyme activity in Korean red pine forests (*Pinus densiflora* Sieb. et Zucc.). This species accounts for approximately 23% of South Korean forest cover and 57% of the nation's coniferous forests, and is the most widely established plantation species (Korea Forest Service 2015). Accordingly, improving the health and productivity of *Pinus densiflora* forests is vital to the current Korean forestry and environmental management (Kim et al. 2012; Kim et al. 2016a). We specifically hypothesized that strong thinning intensity would have greater effects on microbial biomass and enzyme activity than comparatively light thinning intensity because of differences in soil C and N availability. Two *Pinus densiflora* forest sites having different management histories, topographies, and soils were studied to ascertain whether the effects of thinning intensity would be site-specific because of these factors. The effects of thinning intensity were assessed twice, before and after seasonally concentrated precipitation, to account for potential confounding effects of sampling period.

2 Materials and methods

2.1 Study sites

Study sites were two *Pinus densiflora* forests in Jeongseon (site 1: 37° 30′ N, 128° 56′ E) and Gwangneung (site 2: 37°

46′ N, 127° 10′ E), central Korea. The area of each site was 1.5 ha. The average air temperature and annual precipitation in 2015 were 12.4 °C and 676.0 mm near site 1, and 12.4 °C and 988.5 mm near site 2, respectively (Korea Meteorological Administration 2015). Both sites had a temperate climate with seasonally concentrated precipitation from July to August, in which approximately half of annual precipitation occurs (Korea Meteorological Administration 2011). In 2015, precipitation during this period was 273.8 and 469.3 mm near site 1 and 2, respectively (Korea Meteorological Administration 2015). The vegetation was characterized by overstory 51- to 60-year-old *Pinus densiflora* trees and an assortment of herbs, shrubs, and seedlings of broadleaf trees in the understory, such as *Fraxinus rhynchophylla* Hance, *Lindera obtusiloba* Blume, *Quercus serrata* Thunb. ex Murray, *Quercus mongolica* Fisch. ex Ledeb, *Rhus trichocarpa* Miq., *Styrax obassia* Sieb. et Zucc., and *Symplocos chinensis* Druce at site 1 and *Acer pseudosieboldianum* (Pax.) Kom., *Acer barbinerve* Maxim., *L. obtusiloba*, *R. trichocarpa*, *Q. serrata*, *Q. mongolica*, *Smilax china* L., and *Styrax obassia* at site 2 (Ko et al. 2014).

Site 1 was established and managed according to the Korean forestry guidelines. This site was planted at an initial density of 3000 2-year-old seedlings ha^{-1} at 1.8 m × 1.8 m spacing (Korea Forest Research Institute 2012). Within the 80 years of rotation plan, precommercial thinning was performed at ages of 20 and 30 years to remove deformed or poorly growing trees (Korea Forest Research Institute 2012). However, deformed or poorly growing trees were not removed from site 2, which remained in an unmanaged state. In addition, site 2 displayed steeper slopes as well as soils with sandier texture, lower pH, and lower moisture availability, which may limit the productivity of *Pinus densiflora* forests (Lee and Kim 1987; Yoon et al. 2015) (Table 1). These differences in management history and site conditions may have contributed to lower tree density, larger diameter at breast height, and taller tree height at site 1 than those at site 2 (Table 1).

Nine experimental plots, with 8-m radius at site 1 and 10–13-m radius at site 2, were installed at each site. Three treatments were applied each to three plots: un-thinned control (control) and 15% (intermediate thinning) or 30% (heavy thinning) removal of stand basal area. The plots were established in areas with similar slope, aspect, and pre-treatment tree density, and surrounded by a 5-m-wide buffer area to minimize unexpected spatial heterogeneity and edge effect. The thinning treatments, performed in 2008, involved the harvest of stems only, whereas piles of slashes remained and were evenly distributed within the thinned stand. All thinning treatments primarily removed deformed or poorly growing trees having a small diameter at breast height. Hence, thinning of site 2, which contained the larger number of deformed or poorly growing trees, consisted of greater harvest of stems to achieve the target thinning intensity based on basal area, and generated a smaller amount of residue than that of site 1 (Table 1).

Table 1 Characteristics of the control, intermediate thinning (I), and heavy thinning (H) plots in *Pinus densiflora* forest sites

Characteristics[a]	Site 1			Site 2		
	Control	I	H	Control	I	H
Topography						
Altitude (m)	700			500		
Slope (°)	3–24			23–30		
Stand characteristics[b]						
Age (year)	51–60			51–60		
Tree density (tree ha^{-1})	580	473	338	1100	700	313
DBH (cm)	34.6	36.3	38.5	26.3	31.1	43.7
Tree height (m)	21.8	22.5	23.2	14.2	20.7	19.9
Basal area (m^2 ha^{-1})	53.4	48.9	45.4	59.7	53.3	47.1
Thinning residue (Mg ha^{-1})	–	17.9	38.6	–	9.5	13.7
Mineral soil (0–10 cm)[c]						
Soil texture	Loam (sand:silt:clay = 47:45:8)			Sandy loam (sand:silt:clay = 54:36:10)		
Bulk density (g cm^{-3})	0.7 (0.0)	0.6 (0.0)	0.6 (0.0)	0.8 (0.0)	0.7 (0.0)	0.8 (0.0)
Rock content (%)						
Gravimetric basis	5.4 (0.8)	10.0 (1.8)	6.2 (1.3)	8.0 (3.3)	10.2 (2.6)	17.5 (5.5)
Volumetric basis	1.4 (0.2)	2.5 (0.5)	1.3 (0.2)	2.4 (1.2)	3.0 (0.9)	5.5 (1.9)
Porosity (cm^3 cm^{-3})	0.7 (0.0)	0.7 (0.0)	0.7 (0.0)	0.8 (0.0)	0.8 (0.0)	0.8 (0.0)
pH	5.3 (0.0)	5.3 (0.0)	5.2 (0.0)	4.3 (0.1)	4.3 (0.0)	4.3 (0.0)
CEC ($cmol_c$ kg^{-1})	12.0 (0.6)	13.6 (0.7)	12.8 (0.4)	12.9 (0.6)	10.3 (0.3)	11.9 (0.4)

Values in parentheses are the standard errors. Different letters indicate significant differences among the treatments at each site ($n = 3$; $P < 0.05$)

[a] *DBH*, diameter at breast height; *CEC*, cation exchange capacity; *I*, intermediate thinning treatment; *H*, heavy thinning treatment

[b] Data from Ko et al. (2014)

[c] Values were measured 7 years after thinning

2.2 Sampling and processing

The mineral soil at 0–10 cm depth was sampled at each site before (June) and after (September) the seasonally concentrated precipitation, 2015 (7 years after thinning). Soil samples were collected with an auger from 15 random points within each plot to yield one composite soil sample per plot. The composite soil samples were sealed in plastic bags and stored in a refrigerator at 4 °C until analyses. Field-moist subsamples were separated and used for soil water content, microbial

biomass, enzyme activity, and inorganic N analyses, all of which were performed within 2 weeks after sampling. The remaining composite soil samples were air-dried, and sieved through a 2-mm mesh to determine concentrations of total soil C (TSC) and N (TSN) and labile soil C (LSC).

Three additional soil cores (407 cm^3) were collected at 0–10 cm depth from each plot in June, 2015 (7 years after thinning). These samples were air-dried, sieved through a 2-mm mesh, and used to analyze pH, cation exchange capacity (CEC), bulk density, coarse rock content, and texture.

2.3 Soil properties

Soil water content was quantified gravimetrically by drying 15 g of field-moist soil samples at 105 °C. Soil bulk density was calculated by dividing the oven-dry mass of the core samples at 105 °C by the volume of the soil corer. Soil texture was measured by the hydrometer method (Gee and Or 2002). Content of coarse rock (larger than 2 mm) was estimated using both gravimetric (coarse rock mass per total soil mass) and volumetric (coarse rock volume per soil corer volume) bases (Page-Dumroese et al. 1999). Soil porosity was estimated with soil bulk and particle (assumed as 2.65) densities, and soil water saturation was calculated as the percentage of soil water content (converted to volumetric basis using soil bulk density) to porosity (Flint and Flint 2002). Soil pH was measured with a 1:5 soil to water ratio, and CEC was estimated by Brown method (Brown 1943). Soil inorganic N was extracted from 6 g of field-moist soil samples with 30 mL of 2 mol L^{-1} KCl solution. The extracted NH_4^+ was analyzed by the salicylate method (Mulvaney 1996), and the extracted NO_3^- was measured with Griess reagent and VCl_3 reducing agent (Miranda et al. 2001). Soil inorganic N concentration was presented as the sum of NH_4^+ and NO_3^-. To measure TSC and TSN, soil samples were ground with a ball mill for 3–4 h (Nelson and Sommers 1996) and then analyzed by the dry combustion with an elemental analyzer (vario Macro, Elementar Analysensysteme GmbH, Germany). According to the field kit protocol of Weil et al. (2003), LSC was measured by using 0.02 mol L^{-1} $KMnO_4$ solution.

2.4 Microbial biomass

Concentrations of microbial biomass C (MBC) and N (MBN) were determined by the fumigation-extraction method (Brookes et al. 1985; Vance et al. 1987), as modified by other studies (Kim et al. 2017; Perakis and Hedin 2001; Witt et al. 2000). Field-moist samples (8 g) were fumigated with alcohol-free chloroform for 24 h at room temperature and atmospheric pressure. The fumigated samples were aired in a fume hood for at least 2 h to allow the chloroform to evaporate. With 0.5 mol L^{-1} K_2SO_4 solution, C and N in fumigated samples and non-fumigated samples were extracted. Concentrations of C and N in the extracts were quantified with a total organic C analyzer (TOC-V CPH, Shimadzu, Japan). Microbial biomass was estimated by dividing the difference in C or N concentrations between the fumigated and non-fumigated samples with the extraction coefficient (0.45 for MBC and 0.68 for MBN) (Brookes et al. 1985; Joergensen and Mueller 1996).

2.5 Enzyme assays

Enzyme assays were done within 5 days after sampling. To measure enzyme activity, soil suspensions were prepared with 2 g of field-moist samples and 125 mL of 50 mmol L^{-1} NaOAc buffer solution (Saiya-Cork et al. 2002), with the pH adjusted to that of the soil samples within 0.5 units (pH = 5.0 for site 1 and 4.8 for site 2) (DeForest 2009). Homogenization was performed by shaking the suspensions for 30 min and stirring them at high speed for 1 min prior to use in the assays.

Activities of four hydrolytic enzymes: *N*-acetyl-glucosaminidase (NAG), β-glucosidase (BG), cellobiohydrolase (CBH), and β-xylosidase (BX) were analyzed by the fluorometric method (DeForest 2009). For sample assay, 200 μL of the homogenized suspension was incorporated with 50 μL of 4-methylumbelliferone (4-MUB) linked substrate solution into a black polystyrene 96-well microplate. Blanks (250 μL of NaOAc buffer), reference standards (200 μL of NaOAc buffer with 50 μL of 4-MUB solution), negative controls (200 μL of NaOAc buffer with 50 μL of 4-MUB linked substrate solution), quench standards (200 μL of the soil suspension with 50 μL of 4-MUB solution), and sample controls (200 μL of the soil suspension with 50 μL of NaOAc buffer) were also prepared. After 2 h of incubation at 25 °C, 1 mol L^{-1} NaOH solution was added to terminate the reaction. The fluorescence was measured at 355-nm excitation and 460-nm emission levels with a plate reader (Victor 3, Perkin-Elmer, USA). Activities of NAG, BG, CBH, and BX were expressed as μmol 4-MUB kg^{-1} dry soil $hour^{-1}$.

Activities of two oxidative enzymes, phenol oxidase (POX) and peroxidase (PER), were determined by the colorimetric method (DeForest 2009), with some modifications. For sample assay, 2.4 mL of the homogenized suspension was mixed with 0.6 mL L-3,4-dihydroxyphenylalanine (L-DOPA) solution into a transparent polystyrene cuvette. Blanks (3.0 mL of NaOAc buffer), negative controls (2.4 mL of NaOAc buffer with 0.6 mL of L-DOPA solution), and sample controls (2.4 mL of the soil suspension with 0.6 mL of NaOAc buffer) were also prepared. For the total oxidative enzyme assay (the sum of POX and PER activities), 120 μL of 0.3% H_2O_2 solution was also added into the cuvette. After 18 h of incubation at 25 °C, the absorbance of the upper supernatant was measured at 450-nm wavelength with a spectrophotometer (UH5300, Hitachi, Japan). This incubation time was selected for both assays to enable the calculation of

PER activity because PER activity could not be calculated from the total oxidative enzyme activity if different incubation times are used (DeForest 2009). Activities of POX and PER were expressed as μmol L-DOPA kg^{-1} dry soil $hour^{-1}$.

2.6 Statistical analysis

The plot within a treatment at each site in each sampling period was treated as a replicate in statistical analysis ($n = 3$ for each treatment at each site in each sampling period). The enzyme activity data were log-transformed before the statistical analyses for the normalization. The repeated measures ANOVA was performed to assess the effects of thinning in soil properties, microbial biomass, and enzyme activities ($P < 0.05$; Table S1). The post hoc LSD test was applied for the mean separation ($P < 0.05$). Pearson correlation was used to indicate whether microbial biomass and enzyme activities were correlated to soil properties across sites and sampling periods ($P < 0.05$). Linear regression and ANCOVA were used to test whether variation in microbial biomass and enzyme activities at each site in each period was a function of soil properties or purely random ($P < 0.05$). Proc CORR and GLIMMIX procedures of the SAS 9.4 software were used for these statistical analyses.

3 Results

3.1 Soil properties

In June, higher TSC and TSN were recorded under the intermediate and heavy thinning treatments than the control, whereas no difference between treatments was found at site 2 (Table S2). In September, the heavy thinning treatments at sites 1 and 2 had higher TSC and TSN than the control, whereas the intermediate thinning treatment exhibited higher TSC than the control only at site 1 (Table S2). Similarly, LSC and soil water content were generally higher under the intermediate and heavy thinning treatments than the control (Table S2). No difference in the other soil properties, including TSC/TSN ratio, water saturation, and inorganic N, between treatments was detected (Table S2).

3.2 Microbial biomass and enzyme activity

The effect of thinning on MBC differed between the two sites. At site 1, higher MBC was recorded under the intermediate and heavy thinning treatments than the control in June and under the heavy thinning treatment than the control in September (Fig. 1a). At site 2, no difference in MBC between treatments was found (Fig. 1a). Similarly, higher MBN was recorded under the intermediate and heavy thinning treatments than the control in both June and September at site 1,

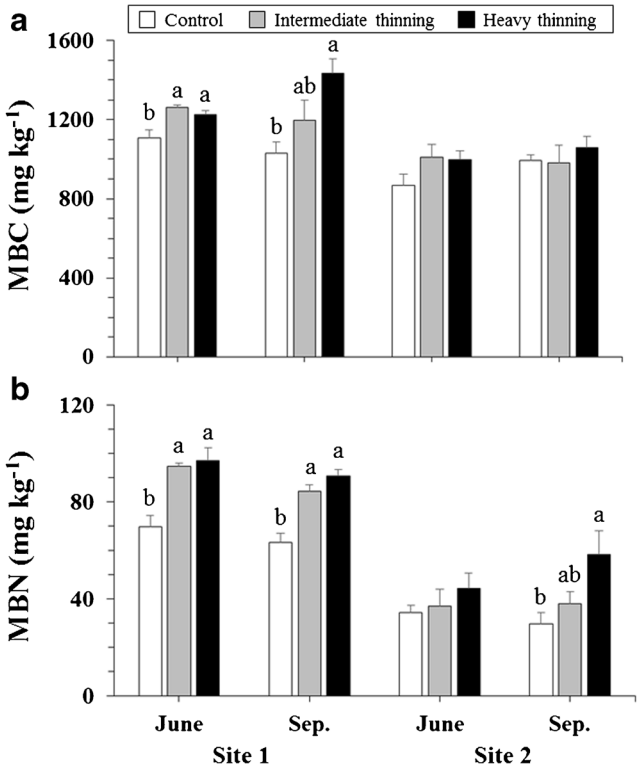

Fig. 1 **a** Microbial biomass C (MBC) and **b** microbial biomass N (MBN) at 0–10 cm soil depth 7 years after thinning. Vertical bars indicate standard errors. Different letters indicate significant differences between the treatments at each site in each sampling period ($n = 3$; $P < 0.05$)

whereas only the heavy thinning treatment in September exhibited higher MBN than the control at site 2 (Fig. 1b). In contrast to MBC and MBN, no difference in NAG, BG, CBH, BX, POX, and PER activities between treatments was found at both sites in both sampling periods (Fig. 2).

3.3 Pairwise relationships

Variation in microbial biomass and enzyme activities was correlated to soil properties (Table 2). Across sites and sampling periods, MBC and MBN were positively correlated to TSC, TSN, soil water content, water saturation, and pH, but negatively correlated to TSC/TSN ratio (Table 2). All hydrolytic enzyme activities were positively correlated to TSC, LSC, and inorganic N, whereas all oxidative enzyme activities were positively correlated to TSC/TSN ratio but negatively correlated to soil water content and pH (Table 2).

In each site and sampling period, TSC and TSN had significant, consistent relationships to MBC and MBN (Fig. 3). Except site 1 in September, MBC was positively related to TSC (Fig. 3a, b). Also, MBN was positively related to TSN in each site and sampling period (Fig. 3c, d). However, the increasing relationship at each site in each sampling period was not detected between enzyme activities and TSC or TSN ($P > 0.05$).

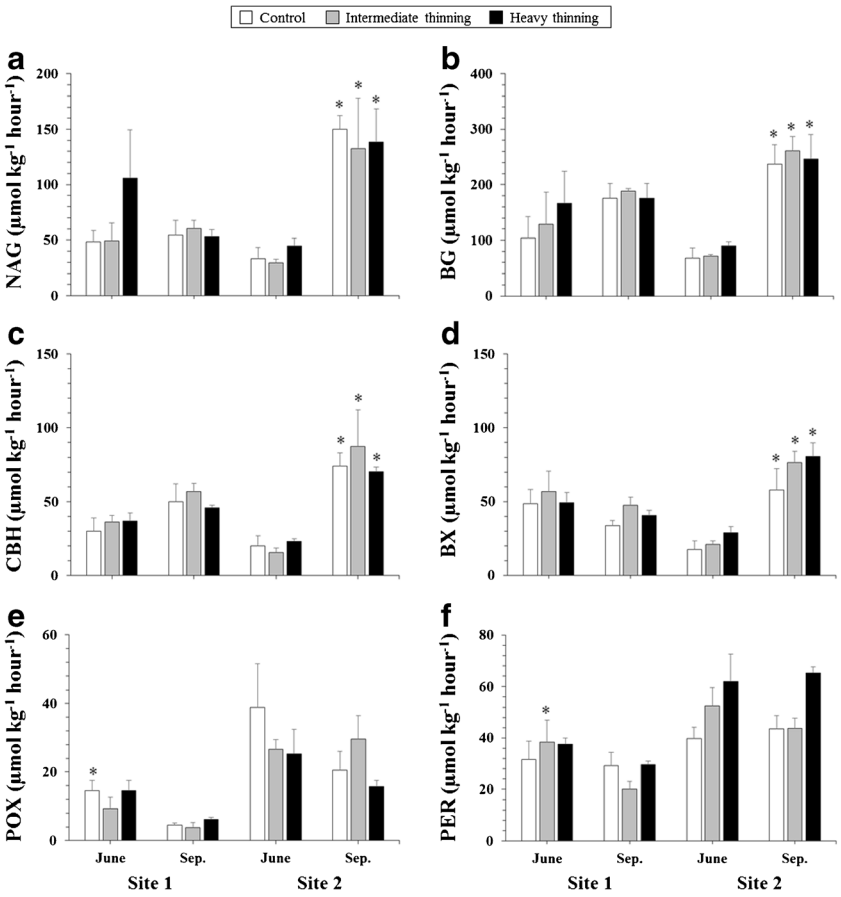

Fig. 2 Soil enzyme activities at 0–10 cm depth 7 years after thinning. Vertical bars indicate standard errors. There were no significant differences between the treatments at both sites in both sampling periods ($n = 3$; $P < 0.05$). Asterisks indicate significant differences between the two sampling periods within each treatment at each site ($n = 3$; $P < 0.05$). **a** *N*-acetylglucosaminidase activity (NAG). **b** β-glucosidase activity (BG). **c** cellobiohydrolase activity (CBH). **d** β-xylosidase activity (BX). **e** phenol oxidase activity (POX). **f** peroxidase activity (PER)

4 Discussion

4.1 The effects of thinning intensity on microbial biomass and enzyme activity

Thinning intensity showed a differential effect on microbial biomass. Especially, highest MBN occurred under the heavy thinning treatment, followed in order by the intermediate thinning treatment and the control at both sites. These findings demonstrate that soil microbes might store more biomass in response to a comparatively heavy thinning intensity over several years (Chen et al. 2015). The increasing patterns in MBN with thinning intensity might be related to shift in substrate availability (Bolat 2014; Chen et al. 2016). At each site, MBN was positively related to TSN (Fig. 3), which also significantly increased with thinning intensity (Table S1). This

Table 2 Correlation coefficients of microbial biomass and enzyme activity with soil properties ($n = 36$; $P < 0.05$)

Variables[a]	TSC	TSN	TSC/TCN	LSC	GWC	WS	Inorganic N	pH
MBC	0.55*	0.73*	− 0.64*	0.15	0.81*	0.48*	0.05	0.67*
MBN	0.48*	0.75*	− 0.80*	− 0.07	0.92*	0.50*	− 0.12	0.86*
NAG	0.42*	0.26	0.17	0.49*	0.08	0.23	0.61*	− 0.24
BG	0.60*	0.43*	0.15	0.58*	0.27	0.23	0.55*	− 0.01
CBH	0.55*	0.39*	0.12	0.53*	0.27	0.23	0.60*	− 0.11
BX	0.72*	0.56*	0.06	0.58*	0.36*	0.25	0.55*	− 0.06
POX	− 0.30	− 0.50*	0.57*	0.06	− 0.69*	− 0.39*	0.05	− 0.67*
PER	− 0.01	− 0.20	0.46*	0.28	− 0.51*	− 0.28	0.19	− 0.66*

[a] *TSC*, total soil C concentration; *TSN*, total soil N concentration; *TSC/TSN*, TSC/TSN ratio; *LSC*, labile soil C concentration; *GWC*, soil gravimetric water content; *WS*, soil water saturation; *MBC*, microbial biomass C concentration; *MBN*, microbial biomass N concentration; *NAG*, *N*-acetyl-glucosaminidase activity; *BG*, β-glucosidase activity; *CBH*, cellobiohydrolase activity; *BX*, β-xylosidase activity; *POX*, phenol oxidase activity; *PER*, peroxidase activity

*Significant at $P < 0.05$

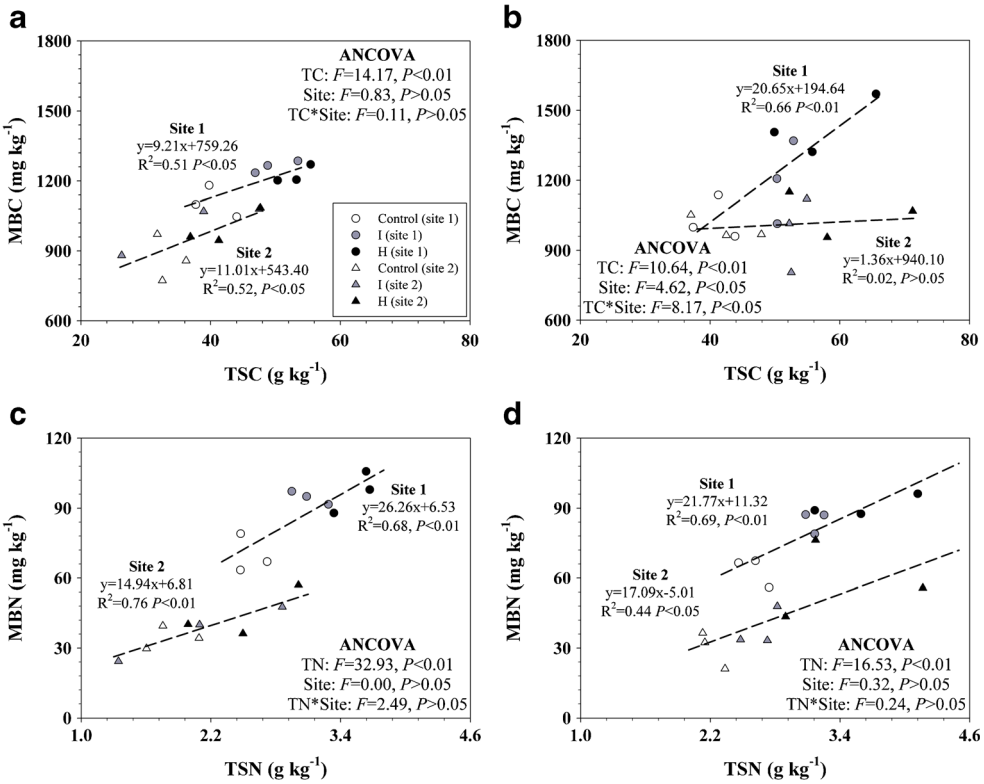

Fig. 3 Relationships of microbial biomass to total soil C and N for the control, intermediate thinning (I), and heavy thinning (H) treatments at site 1 and site 2 in each sampling period ($n = 9$ for each site and period). **a** Relationships between microbial biomass C (MBC) and total soil C (TSC) concentrations in June. **b** Relationships between MBC and TSC in September. **c** Relationships between microbial biomass N (MBN) and total soil N (TSN). **d** Relationships between MBN and TSN in September

pattern might result from the fact that added soil organic C and N could increase microbial biomass by being provided as additional C and N substrates (Benesch et al. 2015) and delaying turnover time of microbial biomass (Wardle 1998).

All thinning intensities had no effect on enzyme activities. This pattern in enzyme activity was consistent with neither higher levels of microbial biomass nor TSC and TSN under the thinning treatments. Our results are unanticipated, given that thinning and residue addition would be expected to stimulate the activity of soil enzymes by adding lignocellulosic and chitin substrates, such as fallen leaves, twigs, and dead roots and mycorrhiza (Adamczyk et al. 2015; Chen et al. 2016; Geng et al. 2012; Wang et al. 2016).

Although it is uncertain why thinning had no effects on enzyme activity, this pattern in enzyme activity might result because of several reasons. For example, thinning increased both LSC and TSC without reduction in inorganic N (Table S2), which might reflect little limitation of C in the soil after thinning. Soil microbes might invest resources for accumulating biomass rather than producing enzymes to acquire further C sources under this condition (Moorhead et al. 2013). In addition, thinning had no effect on TSC/TSN ratio at both sites (Table S2), whereas the increase in TSC/TSN ratio could also contribute to long-term shifts in enzyme activity for N assimilation after thinning (Wang et al. 2016). As soil microbes seek additional N sources for biomass accumulation under increased TSC/TSN ratio conditions (Hodge et al. 2000), the unchanged TSC/TSN ratio might also limit the effects of increased C availability on enzyme activity.

The inconsistency between MBN and enzyme activities reflects that difference between microbial N accumulation and enzymatic N depolymerization might be greater under the heavy thinning intensity than the intermediate thinning intensity and the control. Because the balance between microbial biomass accumulation and enzymatic depolymerization primarily controls N availability to plants (Schimel and Bennett 2004), our results indicate that changes in N availability for the trees remaining after thinning could depend on the implemented thinning intensity. Soil N availability is positively related to aboveground net primary production of *Pinus densiflora* forests (Yoon et al. 2015). Therefore, shift in soil N availability due to thinning intensity might have further differential effects on the forest productivity.

Because microbial biomass is only a gross measure of soil microbes, the present study provides little information regarding microbial species directly mediating C and N cycling (Nannipieri et al. 2003). The lack of information on microbial community could limit our interpretation of the enzyme data, considering that excretion and activation of several enzymes, such as NAG and POX, are dominated by specific microbial groups (Boerner et al. 2008). Therefore, microbial community data should be further obtained and used to support our interpretation of the inconsistency between MBN and enzyme activity. Moreover, our interpretation should be reinforced with the in-site enzyme assays because enzyme activities in the laboratory might not be enough to totally indicate those in the field (Domínguez et al. 2017). Further studies with a larger number of replicate should also be needed to confirm our

explanation for the lack of the effect of thinning on enzyme activities, given the potential confounding effects of the low number of replicate and high heterogeneity between the sites.

4.2 The site-specific patterns in the effect of thinning

One notable result is that the effect of thinning on microbial biomass was stronger at site 1 than at site 2 (Fig. 1), although the magnitude of the basal area removal was similar between the two sites. It might result from the large heterogeneity in soils between the sites. For example, more acidic soils at site 2 might act as environmental stresses and hinder the growth of soil microbes (Wardle 1998). Sandier soil texture and higher rock content at site 2 might generate drier soils by reducing the water holding capacity and provide more unfavorable condition for soil microbes than site 1 (Saxton and Rawls 2006). This divergence in soil moisture availability between the two sites might also be responsible for the site-specific effect of thinning, given that differences in MBN between the thinned and control stands at site 2 increased with soil water content faster than those at site 1 (Fig. 4).

The amount of added residue might also play a role in the site-specific effects of thinning on microbial biomass (Smolander et al. 2015). The intermediate and heavy thinning treatments at site 2, where the first and second smallest amounts of residue (9.5 and 13.7 Mg ha^{-1}) were added, failed to exhibit significant differences in MBC or MBN than in the control in both sampling periods (Fig. 1a, b). Conversely, the heavy thinning treatment at site 1 produced the largest amount of residue (38.6 Mg ha^{-1}) and exhibited significantly higher MBC and MBN than did the control in both sampling periods (Fig. 1a, b). Although the large heterogeneity between the two sites confounds our interpretation of the relationship between the amount of thinning residue and the site-specific effect of thinning, this similarity should be worth being addressed by further studies.

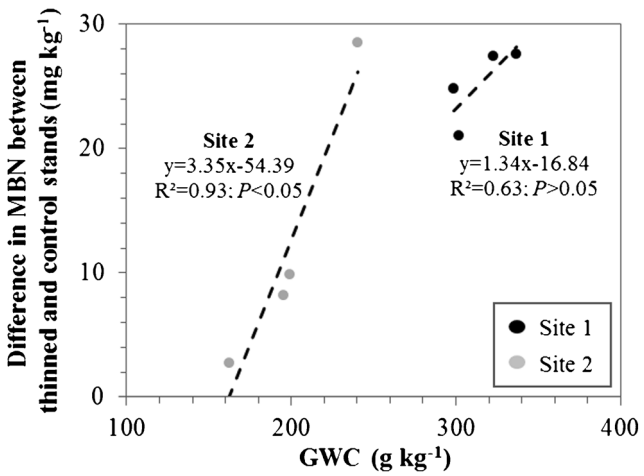

Fig. 4 Relationship of difference in microbial biomass N (MBN) between the thinned and control stands to soil gravimetric water content (GWC) ($n = 4$ for each site). The difference in MBN was calculated by subtracting average MBN of three plots in the control stand from that in the thinned stand at each site in each sampling period

It was also found that the site-specific effect of thinning might occur seasonally. At site 2, MBN under the heavy thinning treatment was higher than the control only after the precipitation period. This might result from the fact that seasonally concentrated precipitation could accelerate the water extraction of biologically available C and N into the soil and reduce hydric stress of soil microbes at site 2 (Benesch et al. 2015; Boyle et al. 2005; Park and Matzner 2003). Inversely, the effect of thinning on MBN was consistently significant in both sampling periods at site 1. The divergence from the pattern at site 1 compared to site 2 might result from differences in the amount of rainfall between the two sites. The amount of rainfall between the two sampling periods at site 1 accounted for only 58% of the amount that occurred at site 2 (see subsection 2.1). It might contribute to the seasonality in the site-specific effect of thinning because low precipitation at site 1 might allow the smaller amount of C, N, and water to infiltrate into the soil than did the greater precipitation at site 2 (Huang et al. 2013; Park and Matzner 2003). Considering the limited number of replicate and high heterogeneity between the two forest sites, our results might not be enough to generalize the site-specific patterns in the effect of thinning across other forests. As such, the site-specific effects of thinning have to be confirmed using a larger number of site replicate and a longer monitoring period.

5 Conclusions

Our results demonstrate that the heavy thinning treatment generally had greater effects on microbial biomass than did the intermediate thinning treatment. The increased level of microbial biomass after thinning corresponded to post-thinning increases in TSC and TSN. Despite these changes in soil properties and microbial biomass, no effect of thinning on enzyme activity was significant at both sites in both sampling periods. It is important to note that the magnitude of the effect of thinning on microbial biomass was lower at site 2, which had sandier, drier, and more acidic soils as well as retained lower amount of thinning residue than site 1. This pattern reflects the importance of considering heterogeneity between sites to interpret the effect of thinning intensity. We expect that our findings will provide information for understanding the contradictory results on microbial biomass and enzyme activity in thinned forests, which have been reported by previous studies (Baena et al. 2013; Chen et al. 2016; Kim et al. 2016c; Maassen et al. 2006).

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